IN VITRO ANTIBACTERIAL ACTIVITIES AND SAFETY OF AQUEOUS EXTRACT OF SELECTED KENYAN MEDICINAL PLANTS AGAINST DIARRHEA CAUSING BACTERIA

Francis Gitau Mugweru (BSc. APD)
I56/11577/2007

A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Infectious Disease Diagnosis) in the School of Pure and Applied Sciences of Kenyatta University

JULY 2016
DECLARATION
I, Francis Gitau Mugweru, duly declare that this thesis is my original work and has not been presented for a degree in any other university or for any other award.

Francis Gitau Mugweru (BSc. APD)
I56/11577/2007

Signature___________________Date__________________

Department of Biochemistry and Biotechnology

We confirm that the work reported in this thesis was carried out by the candidate under our supervision

Supervisors

Prof Joseph JN Ngeranwa
Department of Biochemistry and Biotechnology
Kenyatta University

Signature___________________Date__________________

Prof Eliud NM Njagi
Department of Biochemistry and Biotechnology
Kenyatta University

Signature___________________Date__________________

Prof Peter K Gathumbi
Department of Veterinary Pathology, Microbiology and Parasitology
College of Agriculture and Veterinary Sciences (CAVS)
University of Nairobi

Signature___________________Date__________________
DEDICATION

I dedicate this thesis to my wife Sarah who continually encouraged and prayed for me to complete this research work. Also to my beloved children Cyrus, Lisa and Titus who patiently waited for me to complete my studies.
ACKNOWLEDGEMENT

This work has been achieved with contribution of many people. I am grateful to them all. Some, however, deserve special acknowledgement for the key role they played. I recognize with appreciation the great support and tremendous encouragement from my supervisors, Prof Joseph JN Ngeranwa, Prof Eliud NM Njagi and Prof Peter K Gathumbi, who ensured that all the work went on smoothly and were always available for consultations despite their tight work schedules.

I wish to express my sincere gratitude to Mr. Patrick Mutiso, the taxonomist from Botany Department, University of Nairobi and Mr. Fedinsio Ndegwa of the Department of Pharmacy and Complimentary/Alternative Medicine, Kenyatta University for assisting in field collection and identification of medicinal plants used in this study.

I am greatly indebted to Mr Peter Mbogo, senior technologist at Thika level 5 Hospital and Mr Winfred Gatua, chief technologist at Kenyatta National Hospital for providing the microorganisms used in the study. Thanks to Mr Daniel Maina the Director of Institute of Nuclear Science and Technology, University of Nairobi for the trace element analysis. I acknowledge the assistance by Dr Alfred A Ogao of the Department of Biochemistry and Biotechnology and Mr. James Adino of Department of Medical Laboratory Sciences for supporting me in laboratory analysis and for inspiring me to soldier on with this research.

Lastly, I wish to extend my sincere gratitude to my wife and children for their moral support.
TABLE OF CONTENTS

DECLARATION……………………………………………………………………………… ii
DEDICATION……………………………………………………………………………… iii
ACKNOWLEDGEMENT…………………………………………………………………… iv
TABLE OF CONTENTS……………………………………………………………………… v
LIST OF TABLES…………………………………………………………………………… x
LIST OF PLATES………………………………………………………………………….. xi
ABBREVIATIONS AND ACRONYMS…………………………………………………… xii
ABSTRACT……………………………………………………………………………….. xv

CHAPTER ONE………………………………………………………………………… 1
INTRODUCTION………………………………………………………………………… 1
1.1 Background information…………………………………………………………… 1
1.2 Problem statement…………………………………………………………………… 2
1.3 Justification and significance of the study……………………………………… 3
1.4 Research questions…………………………………………………………………… 3
1.5 General objectives ...................................................................................... 4
1.5 Specific objectives ..................................................................................... 4

CHAPTER TWO……………………………………………………………………... 5
LITERATURE REVIEW………………………………………………………………... 5
2.1 Etiology of diarrhea……………………………………………………………….. 5
2.1.1 Escherichia coli…………………………………………………………………….. 6
2.1.1.1 Pathogenesis………………………………………………………………………. 6
2.1.1.2 Treatment…………………………………………………………………………. 8
2.1.2 Salmonella species………………………………………………………………… 8
2.1.2.1 Treatment and prevention of salmonellosis………………………………… 9
2.1.3 The genus *Shigella*………………………………………………………………………………… 10
2.1.3.1 Treatment……………………………………………………………………………………… 13
2.1.4 *Campylobacter* species…………………………………………………………………………… 13
2.1.4.1 Pathogenesis…………………………………………………………………………………… 14
2.1.4.2 Treatment……………………………………………………………………………………… 14
2.1.5 *Clostridium* species……………………………………………………………………………… 15
2.1.5.1 Pathogenesis………………………………………………………………………………… 15
2.1.5.2 Prevention……………………………………………………………………………………… 16
2.1.5.3 Treatment……………………………………………………………………………………… 16
2.1.6 *Klebsiella* species………………………………………………………………………………… 17
2.1.6.1 Pathogenesis………………………………………………………………………………… 17
2.1.6.2 Treatment……………………………………………………………………………………… 18
2.1.7 *Proteus* species…………………………………………………………………………………… 18
2.1.7.1 Pathogenesis………………………………………………………………………………… 19
2.1.7.2 Treatment and Prevention………………………………………………………………….. 20
2.1.8 *Pseudomonas aeruginosa*……………………………………………………………………… 21
2.1.8.1 Pathogenesis………………………………………………………………………………… 21
2.1.8.2 Diagnosis……………………………………………………………………………………… 23
2.1.8.3 Resistance of *Pseudomonas* to antimicrobial drugs…………………………………… 22
2.1.8.4 Treatment……………………………………………………………………………………… 23
2.2 Medicinal plants and traditional medicine……………………………………………………… 23
2.2.1 *Boscia angustifolia* (A. Rich.) Capparaceae………………………………………………… 24
2.2.2 *Lantana trifolia* (L.) Verbenaceae…………………………………………………………… 25
2.2.3 *Senna spectabilis* (L.) Caesalpiniaceae……………………………………………………… 26
2.2.4 *Maytenus putterlickioides Loes* (A. Rich.) Celastraceae………………………………… 26
2.2.5 *Olinia usambarensis* (Gilg.) Oliniaceae .................................................. 26

2.3 Phytochemical in medicinal plants .......................................................... 28

CHAPTER THREE .......................................................................................... 31

MATERIALS AND METHODS ........................................................................ 31

3.1 Collection of medicinal plants .................................................................. 31

3.2 Processing and extraction ......................................................................... 31

3.3 Bacterial stocks ......................................................................................... 32

3.4 Comparative studies ................................................................................ 32

3.5 Determination of susceptibility of bacterial isolates to plant extracts ......... 33

3.6 Determination of minimum inhibitory concentration (MIC) ................. 35

3.7 Determination of Minimum bactericidal concentration (MBC) ............. 36

3.8 *In vivo* toxicity testing .......................................................................... 37

3.8.1 Experimental animals ........................................................................... 37

3.8.2 Histopathology ..................................................................................... 38

3.8.3 Biochemical assays .............................................................................. 39

3.8.4 Determination of hematological parameters ....................................... 40

3.9 Qualitative Phytochemical screening ...................................................... 41

3.9.1 Testing for alkaloids ............................................................................. 41

3.9.2 Testing for tannins .............................................................................. 42

3.9.3 Testing for anthraquinones ................................................................. 43

3.9.4 Test for triterpenes and steroids ......................................................... 42

3.9.5 Test for saponins ................................................................................ 42

3.9.6 Testing for flavonoids ......................................................................... 43

3.9.7 Test for coumarins ............................................................................. 43

3.9.8 Test for reducing sugars ................................................................. 43
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.10</td>
<td>Determination of mineral element content</td>
<td>44</td>
</tr>
<tr>
<td>3.11</td>
<td>Data management and statistical analysis</td>
<td>45</td>
</tr>
<tr>
<td><strong>CHAPTER FOUR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RESULTS</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>Preliminary Screening of Plant Extracts</td>
<td>46</td>
</tr>
<tr>
<td>4.1.1</td>
<td>Yield of plant extracts</td>
<td>46</td>
</tr>
<tr>
<td>4.1.2</td>
<td>Antibiotics Susceptibility Testing</td>
<td>46</td>
</tr>
<tr>
<td>4.1.3</td>
<td>Susceptibility of Bacterial Isolates to Plant Extracts</td>
<td>50</td>
</tr>
<tr>
<td>4.2</td>
<td>Minimum inhibitory concentrations (MICs) and minimum bactericidal</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>concentrations (MBCs) of <em>S. spectabilis</em>, <em>M. putterlickioides</em>, and</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>O. usambarensis</em> aqueous extracts</td>
<td></td>
</tr>
<tr>
<td>4.3</td>
<td>Phytochemical Composition of selected plant extracts</td>
<td>55</td>
</tr>
<tr>
<td>4.4</td>
<td>Mineral concentration in μg/g in the plant extracts, mineral elements</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>intake in μg/day/mouse and Recommended Daily allowance μg/day/mouse</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td><em>In vivo</em> Toxicity Studies</td>
<td>57</td>
</tr>
<tr>
<td>4.5.1</td>
<td>Effects of oral administration of aqueous plant extracts at 1g /kg</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>body weight daily for 28 days on weekly body weights and average</td>
<td></td>
</tr>
<tr>
<td></td>
<td>weekly body weight change in mice</td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td>Effects of oral administration of aqueous plant extracts at 1g /kg</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>body weight daily for 28 days on the percent relative organ to body</td>
<td></td>
</tr>
<tr>
<td></td>
<td>weights in mice</td>
<td></td>
</tr>
<tr>
<td>4.7</td>
<td>Effect of oral administration of aqueous plant extracts at 1g /kg</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>body weight daily for 28 days on the hematological parameters in mice</td>
<td></td>
</tr>
<tr>
<td>4.8</td>
<td>Effects of oral administration of aqueous plant extracts at 1g /kg</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>body weight daily for 28 days on the differential white blood cell</td>
<td></td>
</tr>
<tr>
<td></td>
<td>count (DLC) in mice</td>
<td></td>
</tr>
<tr>
<td>4.9</td>
<td>Effect of oral administration of aqueous plant extracts at 1g /kg</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>body weight</td>
<td></td>
</tr>
</tbody>
</table>
daily for 28 days on the biochemical parameters in mice.................

4.10  Histopathology........................................................................ 65
4.10.1 The Liver............................................................................ 65
4.10.2 Kidney.................................................................................. 67
4.10.2 Intestine............................................................................... 68

CHAPTER FIVE.................................................................................. 71

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS..................... 71

5.1 DISCUSSION.............................................................................. 71
5.2 CONCLUSIONS.......................................................................... 83
5.3 RECOMMENDATIONS............................................................... 83
5.4 SUGGESTIONS FOR FURTHER RESEARCH.............................. 84

REFERENCES.................................................................................. 86

APPENDICES................................................................................... 99

APPENDIX 1.................................................................................... 99

APPENDIX 2...................................................................................... 101

APPENDIX 3...................................................................................... 103
LIST OF TABLES

Table 4.1 Yield of aqueous plant extracts per 100g of plant material. 46
Table 4.2 Zone of inhibition (millimeters) of respective antibiotics against bacterial isolates. 49
Table 4.3 Zone of inhibition (millimeters) of aqueous extracts of selected plants against bacterial isolates. 52
Table 4.4 Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of the plant extracts. 54
Table 4.5 Phytochemical determination of selected plant extracts. 55
Table 4.6 Mineral concentration in µg/g in the plant extracts, mineral elements intake in µg/day/mouse and Recommended Daily allowance µg/day/mouse. 56
Table 4.7 Effects of oral administration of aqueous plant extracts at 1g /kg body weight daily for 28 days on weekly weights of mice. 57
Table 4.8 Effects of oral administration of aqueous plant extracts at 1g /kg body weight daily for 28 days on percent relative organ to body weights in mice. 59
Table 4.9 Effects of oral administration of aqueous plant extracts at 1g /kg body weight daily for 28 days on hematological parameters in mice. 60
Table 4.10 Effects of oral administration of aqueous plant extracts at 1g /kg body weight daily for 28 days on differential white blood cell count in mice. 62
Table 4.11 Effects of oral administration of aqueous plant extracts at 1g /kg body weight daily for 28 days on biochemical parameters in mice. 64
## LIST OF PLATES

<table>
<thead>
<tr>
<th>Plate</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Photomicrograph of a histological section of a liver of a mouse orally treated with normal saline, for 28 days</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>Photomicrograph of a histological section of a liver of a mouse orally treated with an aqueous extract of <em>Olinia usambarensis</em> (1g/kg bw/day) for 28 days</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>Photomicrograph of a histological section of a liver of a mouse orally treated with an aqueous extract of <em>M. putterlickioides</em> (1g/kg bw/day) for 28 days</td>
<td>66</td>
</tr>
<tr>
<td>4</td>
<td>Photomicrograph of a histological section of a liver of a mouse orally treated with an aqueous extract of <em>Senna spectabilis</em> (1g/kg bw/day) for 28 days</td>
<td>66</td>
</tr>
<tr>
<td>5</td>
<td>Photomicrograph of a histological section of a kidney of a mouse orally treated with normal saline, food and water for 30 days</td>
<td>67</td>
</tr>
<tr>
<td>6</td>
<td>Photomicrograph of a histological section of a kidney of a mouse orally treated with an aqueous extract of <em>M. putterlickioides</em> (1g/kg bw/day) for 28 days</td>
<td>67</td>
</tr>
<tr>
<td>7</td>
<td>Photo-micrograph of a histological section of a kidney of a mouse orally treated with an aqueous extract of <em>Senna spectabilis</em> (1g/kg bw/day) for 28 days</td>
<td>68</td>
</tr>
<tr>
<td>8</td>
<td>Photo-micrograph of a histological section of a kidney of a mouse orally treated with an aqueous extract of <em>O. usambarensis</em> (1g/kg bw/day) for 28 days</td>
<td>68</td>
</tr>
<tr>
<td>9</td>
<td>Photo-micrograph of a histological section of intestine of a mouse orally treated with normal saline, food and water for 28 days</td>
<td>69</td>
</tr>
<tr>
<td>10</td>
<td>Photomicrograph of a histological section of intestine of a mouse orally treated with an aqueous extract of <em>Senna spectabilis</em> (1g/kg bw/day) for 28 days</td>
<td>69</td>
</tr>
<tr>
<td>11</td>
<td>Photomicrograph of a histological section of intestines of a mouse orally treated with an aqueous extract of <em>Senna spectabilis</em> (1g/kg bw/day) for 28 days</td>
<td>69</td>
</tr>
<tr>
<td>12:</td>
<td>Photomicrograph of a histological section of intestines of a mouse orally treated with an aqueous extract of <em>O. usambarensis</em> (1g/kg bw/day) for 28 days</td>
<td>70</td>
</tr>
</tbody>
</table>
# ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>Alanine phosphatase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine aminotransferase</td>
</tr>
<tr>
<td>AMP</td>
<td>2-amino-2-methyl-1-propanol</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate aminotransferase</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine-5-triphosphate</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type culture Collection</td>
</tr>
<tr>
<td>BUN</td>
<td>Blood urea Nitrogen</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>Cu</td>
<td>Copper</td>
</tr>
<tr>
<td>CK</td>
<td>Creatine kinase</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming units</td>
</tr>
<tr>
<td>DLC</td>
<td>Differential lymphocytes count</td>
</tr>
<tr>
<td>DST</td>
<td>Diagnostic Sensitivity Test Agar</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>Fe</td>
<td>Iron</td>
</tr>
<tr>
<td>fL</td>
<td>femtolitres</td>
</tr>
<tr>
<td>GDH</td>
<td>Glutamate dehydrogenase</td>
</tr>
<tr>
<td>GLP</td>
<td>Good Laboratory Practice</td>
</tr>
<tr>
<td>HCT</td>
<td>Hematocrit</td>
</tr>
<tr>
<td>HGB</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>HK</td>
<td>Hexokinase</td>
</tr>
<tr>
<td>K</td>
<td>Potassium</td>
</tr>
<tr>
<td>KEMRI</td>
<td>Kenya Medical Research Institute</td>
</tr>
<tr>
<td>KNH</td>
<td>Kenyatta National Hospital</td>
</tr>
</tbody>
</table>
LDH  Lactate dehydrogenase
Ly   Lymphocytes
NCTC National Collection of Type Culture
Mn   Manganese
Mo   Monocytes
MBC  Minimum Bactericidal Concentration
MCV  Mean corpuscular volume
MCH  Mean corpuscular hemoglobin
MCHC Mean corpuscular hemoglobin concentration
MCA  Multi-channel analyzer
MDH  Malate dehydrogenase
MI   Myocardial infarction
MIC  Minimum Inhibitory Concentration
MHA  Muller Hinton agar
MHB  Muller Hinton broth
MPV  Mean platelet volume
Pb   Lead
PCT  Plateletcrit
PCV  Packed cell volume
PDW  Platelet cell distribution width
PLT  Platelet count
PPM  Parts per million
RBC  Red blood cells
RDW  Red blood cell distribution width
STP  Society of Toxicological Pathology
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti</td>
<td>Titanium</td>
</tr>
<tr>
<td>TXRF</td>
<td>Total reflection X-ray Fluorescence system</td>
</tr>
<tr>
<td>V/V</td>
<td>Volume by volume</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cells</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
**ABSTRACT**

Diarrheal diseases constitute a major public health problem, particularly in the developing world, where mortality and morbidity rates are still very high. Acute diarrhea is a common cause of death in developing countries and second most common cause of infant deaths worldwide. Diarrhea is a very common complication of infection with the HIV and often leads to wasting and malnutrition. In developing countries, up to 80% of children and 90% of adults with HIV infection develop diarrhea. Many of the antibiotics used in management of diarrhea caused by bacteria are experiencing increased resistance posing a great public health concern. This calls for the need to continue searching for new drugs to control this condition. In this study, aqueous extracts of five selected medicinal plants were investigated for antibacterial activity against diarrhea causing bacteria pathogens (*Salmonella* spp., *Campylobacter* spp., *Shigella* spp., *Diarrheagenic E. coli*, *Pseudomonas aeruginosa*, and *Proteus* spp.). The clinical isolates and standard organisms were obtained from reliable laboratories of KEMRI and KNH. Identities of the micro-organisms were confirmed by colonial morphology, gram staining and biochemical test. The plants were collected within Kenya from their ecological zones. They were selected from among medicinal plants used in the treatment of various ailments using the information obtained from ethno-medical practices and literature. Aqueous extraction and freeze drying was employed in preparation of plant extracts. Disc diffusion technique was used for preliminary determination of *in vitro* antibacterial activity of the extract by evaluating the ability to inhibit the growth of the bacterial species. Three out of the five selected medicinal plants extracts tested by disk diffusion technique had inhibitory activity on most bacterial isolates with inhibition diameter ranging from 9mm to 18mm. These are *Senna spectabilis* (Leaves), *Maytenus putterlickioides* (Roots) and *Olinia usambarensis* (Leaves). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts with the most prominent activity were evaluated by plate dilution method. The efficacy and potency of the extracts were assessed by comparing the MIC and MBC values of the five bacterial species to the selected medicinal plant extract with those of chloramphenicol. A regression analysis was used to analyze mean MIC and MBC for each of the selected organisms. All the extracts exhibited an MBC range of 12.5 to 75mg/ml that was greater than MIC range of 6.25 to 50mg/ml, which indicates a bacteriostatic activity. This was similar to chloramphenicol, suggesting the mode of action may be closely related. *In vivo* toxicity of the plant extracts was assessed using mice model. The reduced growth rate, increased the percent relative organ to body weights and increased levels of some serum parameters in mice treated with plant extracts (*S. spectabilis* and *O. usambarensis*) relative to that of control indicates some toxic effects. This study will enhance understanding of efficacy and safety of ethno-medical materials in the management of diarrhea caused by bacterial pathogens. It will also promote possible scientific development of antibacterial agents from these plants.
CHAPTER ONE

INTRODUCTION

1.1 Background information

Diarrhea is one of the leading causes of morbidity and mortality among children under five years in the developing world (Moyo, 2007). During the period from 1950 to 1970s, it was estimated that 4.6 million children died annually from diarrhea in the developing world (Parashar, 2003). Mortality due to diarrhea declined to approximately 3.3 million annually in the 1980s (Moyo, 2007). Total deaths from diarrhea are estimated at 1.26 million in 2013 – down from 2.58 million in 1990. In 2012, diarrhea was recorded as the second most common cause of deaths in children younger than five where 0.76 million died in the developing world (Abdelmalak and John, 2013). Despite the decline in mortality in most developing countries, diarrhea still remains one of the principal causes of morbidity in the developing world, with each child experiencing an average of three episodes of diarrhea per year (Abdelmalak and John, 2013). In these countries, diarrheal diseases are the second most common illnesses of children after acute respiratory illness. Other long term problems that can result include stunted growth and poor intellectual development (Zwane and Kremer, 2007). In developing countries, up to 80% of children with HIV infection develop diarrhea (Liu et al., 2012).

A number of studies have focused on bacterial causes of diarrhea in AIDS patients. *Salmonella spp, Campylobacter spp, Shigella spp, diarrheagenic E. coli, Clostridium difficile* and *Mycobacterium avium* complex are some of the bacteria that have been isolated in AIDS patients (Tate et al., 2012). Among the bacterial causes, diarrheagenic *Escherichia coli* (DEC) is the most important etiologic agent of
childhood diarrhea and represents a major public health problem in developing countries (Al-Gallas et al., 2006).

Transmission of diarrheagenic *Escherichia coli* is mainly associated with poor sanitation and hygienic conditions (WHO, 2006). If the bacteria that cause opportunistic infections acquire resistance to commonly used antibiotics, they become difficult to treat (Spellberg et al., 2008). In today’s battle against infectious agents, conventional medicine may be effective. However, due to the high cost of drugs and increasing bacterial resistance to these drugs, alternative medicine seems to be the latest and the future weapon. As a result, a growing number of health care consumers are turning to plant derived medicines which are cheaper, believing that these products are effective and also safe (Pitman, 2005). However, this may not be the case as most of the plant products in use have not been taken through any scientific evaluation. Selected medicinal plants such as *Maytenus putterlickioides loes*, *Boscia angustifolia*, *Lantana trifolia*, *Senna spectabilis* and *Olinia usambarensis* are known to cure diabetes and have antibacterial activity (Hailu et al., 2005).

1.2 Problem statement

Diarrhea is one of the leading causes of morbidity and mortality among children under the age of five years in the developing world (Moyo, 2007). Opportunistic bacteria have been identified as the major cause of diarrhea in HIV infected patients (Bernstein et al., 1995). Treatment of these emerging and re-emerging strains of diarrhea causing bacteria has become difficult due to their increased tolerance to the available antibiotics. The medicinal uses of the five selected plants in this study are documented (Kokwaro, 1976). However, there is no record in the literature of the
antibacterial activity and toxicity profiles of these plants. The conventional drugs for
treatment of diarrhea causing bacteria are costly, unaffordable and inaccessible
especially in the rural and remote regions of Kenya. New therapeutic agents with
different mode of antibacterial action are therefore desired.

1.3 Justification and significance of the study

The use of plants and plant products in disease control has persisted despite advances
in the modern pharmaceutical products and dominance of synthetic drugs all over the
world. The pharmacological effects of these selected plants have not been subjected to
rigorous trials and control. Thus these claims remain unsubstantiated. There is
therefore lack of credibility, awareness and laboratory data to support most of the
reported therapeutic claims. There is also need for continuous development of new
antibacterial agents for treatment of diarrhea with known potential traditional herbs to
keep in check the growing resistance of bacteria to common conventional drugs.

There is need to identify and develop alternative drugs, which are effective, affordable
and easily accessible to diarrhea patients. This study was aimed at determining the
antibacterial potential of the selected medicinal plants and their safety in the treatment
of diarrhea caused by bacteria. It will complement efforts in the search for new and
effective drugs. This study will enhance understanding of the efficacy and safety of
ethno-medical materials in the management of diarrhea. Additionally it will provide
stimulus to the utilization and development of indigenous knowledge in health care.

1.4 Research questions

1. What is the effect of aqueous extracts of the selected medicinal plants on
targeted diarrhea-causing bacteria?
2. What is the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous extracts of the selected medicinal plant against targeted diarrhea-causing bacteria?

3. What is the qualitative phytochemical composition and quantitative mineral element content of the selected medicinal plants?

4. Do the aqueous extracts of the selected medicinal plants have toxic effects in Swiss albino mice models?

**1.5 General objective**

To determine the antibacterial activities and safety of aqueous extracts of the selected medicinal plants against targeted diarrhea causing bacteria.

**1.5.1 Specific objectives**

1. To determine the effects of aqueous extracts of the selected medicinal plants on the targeted diarrhea-causing bacteria by disk diffusion method.

2. To determine the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous extracts of the selected medicinal plant against targeted diarrhea-causing bacteria.

3. To determine qualitative phytochemical composition and quantitative mineral element content of the aqueous extracts of the selected medicinal plants.

4. To determine in vivo preliminary toxicity/safety of aqueous extracts of the selected medicinal plants in Swiss albino mice models.
CHAPTER TWO
LITERATURE REVIEW

2.1 Etiology of diarrhea

Diarrhea is passing loose stool more frequently (three or more times a day) than normal. Acute diarrhea is a common problem that usually lasts 1 or 2 days and goes away on its own. Diarrhea that lasts at least 4 weeks may be a symptom of a chronic disease. Diarrhea of any duration may cause dehydration, which means the body lacks enough fluid and electrolytes, including sodium, potassium, and chloride hence fails to function properly (Ramakrishna, 1999). Dehydration due to loss of water and electrolytes is the cause of death in diarrheic patients. Oral rehydration salts (ORS) and oral rehydration therapy (ORT) is successful in managing diarrhea among children. Antidiarrheal agents such as loperamide, diphenoxylate- atropine, and tincture of opium can be given to the patients (Wilcox, 2000).

Acute diarrhea is usually caused by a bacterial, viral, or parasitic infection. Chronic diarrhea is usually related to a functional disorder such as irritable bowel syndrome or an intestinal disease such as Crohn’s disease. Many viruses cause diarrhea, including rota virus, noro virus, cytomegalovirus, herpes simplex virus, and viral hepatitis. Infection with the rota virus is the most common cause of acute diarrhea in children under five years old. Rota virus diarrhea usually resolves in 3 to 7 days but can cause problems in digestion of lactose for up to a month or longer (Greenberg and Estes, 2009). Parasites that cause diarrhea include Giardia lamblia, Entamoeba histolytica, and Cryptosporidium (Kiser et al., 2008). Inflammatory bowel disease, ulcerative colitis, Crohn’s disease, and celiac disease often lead to diarrhea where some people have difficulty in digesting certain ingredients, such as lactose, the sugar found in
milk and milk products resulting to diarrhea. Others may have diarrhea if they eat certain types of sugar substitutes in excessive quantities. Stomach surgery, result in some people developing diarrhea, which may cause quick movement of food through the digestive system. Antibiotics, cancer drugs, and antacids containing magnesium can all cause diarrheas (DuPont et al., 2009). Several types of bacteria ingested through contaminated food or water can cause diarrhea. The common bacteria that cause diarrhea include *Campylobacter, Salmonella, Shigella, Escherichia coli* and *Clostridium perfrigen* among others (Viswanathan et al., 2009). In the elderly, particularly those who have been treated with antibiotics for unrelated infections, a toxin produced by *Clostridium difficile* often causes severe diarrhea (Rupnik et al., 2009).

### 2.1.1 *Escherichia coli*

*Escherichia coli* are Gram negative, facultative and anaerobic bacteria, commonly found in the lower intestine of warm blooded organisms. The bacteria are non-motile or motile by peritrichous flagella (Schlievert, 1993). This bacterium is a major facultative inhabitant of the large intestine. It is one of the most frequent causes of some of the common bacterial infections, including, cholecystitis, bacteremia, cholangitis, urinary tract infections (UTI), and traveler’s diarrhea (Steffen, 2005).

#### 2.1.1.1 Pathogenesis

*Escherichia coli* accounts for 28.5% of the vast majority cases of neonatal meningitis. Pregnant women are at a higher risk of colonization with the K1 capsular antigen strain of *E. coli*. This strain is also commonly observed in neonatal sepsis. The mortality rate is 8%, and most survivors have subsequent neurologic or developmental
abnormalities. Respiratory tract infections due to *E. coli* are uncommon and are almost always associated with *E. coli* urinary tract infections (UTI). No virulence factors have been implicated. However, *E. coli* pneumonia can also be community-acquired in patients who are immunosuppressed (Bradley, 2002).

Six strains of diarrheagenic *E. coli* have been reported and are associated with 25% of acute diarrhea in immunocompetent individuals (Okeke *et al.* 2003). Enterotoxigenic *E. coli* (ETEC) is a cause of traveler’s diarrhea. Enteropathogenic *E. coli* (EPEC) is a cause of childhood diarrhea (Mandell *et al.* 2000). Enteroinvasive *E. coli* (EIEC) causes Shigella-like dysentery. Enterohemorrhagic *E. coli* (EHEC) causes hemorrhagic colitis or hemolytic uremic syndrome (HUS). Enteroaggregative *E. coli* (EAggEC) is primarily associated with persistent diarrhea in children in developing countries, and enteroadherent *E. coli* (EAEC) is a cause of childhood diarrhea and also traveler’s diarrhea in Mexico and North Africa. ETEC, EPEC, EAggEC, and EAEC colonize the small bowel, while EIEC and EHEC preferentially colonize the large bowel prior to causing diarrhea. *E. coli* is a leading cause of nosocomial infections originating from gastro-intestinal and genito-urinary tracts (Gaynes and Edwards, 2005).

Serotype *Escherichia coli* 0157:H7 causes food poisoning in human mainly through consumption of contaminated food (meat and raw milk) and water. In Africa, Gassama *et al.* (2004), found a positive correlation between chronic diarrhea and isolation of *E. coli* from the intestinal mucosa of AIDs patients. Patients infected with these bacteria have fever, bloody diarrhea and polymorphonuclear cells in the stool (Foley *et al.*, 2009).
2.1.1.2 Treatment

*Escherichia coli* organisms are resistant to multiple antibiotics (Mandell *et al.*, 2000). This is thought to be a plasmid-mediated property. The choice of a specific antimicrobial agent depends on local susceptibility patterns. Once bacteremia is confirmed, treatment may be modified. *Escherichia coli* are sensitive to sulphonamides, cephalosporins, ciprofloxacin, chloramphenicol, aminoglycoside, ampicillin and tetracycline (Tesfaye *et al.*, 2009). *E coli* enteric infections require fluid replacement with solutions containing appropriate electrolytes. Antimicrobials known to be useful in cases of traveler's diarrhea include doxycycline, trimethoprim/sulfamethoxazole (TMP/SMZ), fluoroquinolones, and rifaximin. They shorten the duration of diarrhea by 24-36 h. Antibiotics are not useful in enterohemorrhagic *E. coli* (EHEC) infection and may predispose to development of Hemolytic uremic Syndrome (HUS). Antibiotic agents are contraindicated in children and in persons with enteroinvasive *E. coli* (EIEC) infection (Johnson, 2010).

2.1.2 *Salmonella species*

*Salmonella* is a genus of rod-shaped Gram-negative, non-spore forming, motile and facultative anaerobic bacteria of the family Enterobacteriaceae. They are non-lactose fermenters though lactose fermenters have been isolated (Easterlin *et al.*, 1969). The genus has 3 serotypes: *S. enterica*, *S. bongori* and *S. subterranea*. The majority of disease causing serovars is from subspecies 1 which includes the serovars *Typhimurium* and *Typhi*. Almost all the members of the genus *Salmonella* are potentially pathogenic and are common inhabitants of the intestinal tracts of many animals especially poultry and cattle. Under unsanitary conditions, they can cause food poisoning (Tortora *et al.*, 2001). Based on three major antigens flagella (H),
somatic (O), and virulence (Vi), there are over 2000 different serotypes of *Salmonella*, represented as a single species *Salmonella enterica* (Greenwood *et al.*, 2002). The serotype *S. typhi* and *S. paratyphi* only colonizes humans while non-typhi *Salmonella* can infect a wide variety of animal hosts. In human, *Salmonella* can cause a wide spectrum of clinical illness though there are four major syndromes: enteric fever, gastro-enteritis, bacterium with or without metastatic infection and the asymptomatic carrier (Greenwood *et al.*, 2002).

Salmonellosis or Salmonella gastroenteritis is the disease caused by Salmonella spp. Food products such as poultry, eggs and unpasteurized milk are the largest source of human infection. Person-to-person transmission occur via the fecal-oral route. Salmonellosis or Salmonella gastroenteritis outbreaks have occurred in hospitals and day baby care centers (Mandell *et al.*, 1995). The infection has an incubation period of about 12-36 hours and an infectious dose of $10^6$ to $10^9$ organisms, depending on the serotype required for infection (Tortora *et al.*, 2002). The pathogens infect the intestinal mucosa but may pass through the mucosa to enter the lymphatic and cardiovascular systems and may spread to affect many other organs. The symptoms of salmonella gastroenteritis include fever, nausea, abdominal pain and cramps, and diarrhea.

2.1.2.1 Treatment and prevention of salmonellosis

In the immune-competent patients diarrhea caused by *Salmonella* is self-limiting. Antibiotic therapy is usually not recommended as it does not significantly improve symptoms and may increase relapse rate (Miller, 1998). Management may include replacement of fluids and electrolytes and control of nausea, vomiting and pain. Drugs
to control the higher motility of the gut are contra-indicated; they may give symptomatic relief for a while, but may transform a trivial gastro-enteritis into a life-threatening bacteremia by paralyzing the bowel. Antibiotics have no part to play in the management in most cases, but when there is increased risk of bacteremia or invasion, an antibiotic may protect against these complications (Greenwood et al. 2002).

Given the increased severity potential for extra intestinal spread, and the high relapse rate, salmonellosis requires treatment in HIV-infected persons. Drugs used to treat salmonella infections include chloramphenicol, ampicillin, amoxillin and trimethoprim-sulfamethoxazole. Ciprofloxacin has emerged as the drug of choice for the treatment of adult typhoid and is proving effective and free from side effects in children (Greenwood et al., 2002). Antibiotic therapy should, however, be guided by antimicrobial susceptibilities since multidrug resistance Salmonella have been reported (Kariuki et al., 2000; John et al., 2003). Prevention depends on good sanitation practices to deter contaminations and on proper handling of foods of animals’ origin. People should be educated regarding the risks of eating uncooked or inadequately cooked food such as eggs, poultry, meat, sea food and unpasteurized dairy products. Exposure to pets including reptiles has also been associated with salmonellosis and potential risks should be explained (Leonard et al., 2005).

2.1.3 The genus Shigella

The genus Shigella is subdivided on biochemical and serological grounds into four species: S. dysenteriae, S. flexineriae, S. boydii and S. sonnei. They are members of the family Enterobacteriaceae and are closely related to the genus Escherichia. By use
of their antigens, strains of *S. dysenteriae* can be divided into 13, *S. boydii* 18 and *S. flexineriae* into 6 serotypes. Strains of *S. sonnei* are serologically homogeneous (Greenwood *et al.*, 2002).

Microscopically, in stained preparations, *Shigella* is Gram-negative, non-spore forming rod-shaped, non-motile and non-capsulated bacteria. Culturally they are similar to most other enterobacteria and like the members of the genus *Salmonella*, they do not ferment lactose following overnight incubation. However *S. sonnei* ferments lactose slowly (Itoh *et al.*, 1991).

Shigellosis, also called bacillary dysentery is a food borne illness caused by members of the genus *Shigella*. Shigellosis rarely occurs in animals other than human and primates such as monkeys and chimpanzees. The causative organism is frequently found in water polluted with human feces, and is transmitted via the fecal-oral route. The usual mode for transmission is directly person-to-person, hand to mouth in the setting of poor hygiene among children. Ten to fifteen (10 to 15) percent of infected people will die (WHO, Shigellosis, 2007).

Infection with *Shigella* results in the destruction of epithelial cells of the intestinal mucosa in the cecum and rectum. Some strains produce enterotoxins and shiga toxins similar to verotoxin of *E. coli* 0157:H7 associated with causing hemolytic uremic syndrome (HUS) (Hale and Keusch, 1996). *Shigella* is implicated as one of the pathogenic cause of reactive arthritis worldwide (Hill and Lillicrap, 2003). The severity of the illness is mostly associated with the species involved where *S. sonnei* causes relatively mild dysentery while infection with *S. dysenteriae* results in severe
dysentery (Tortora et al., 2001). The incubation is usually between 2 and 3 days but may be as short as 12 hours. Abdominal pain is the initial symptom followed by an onset of watery diarrhea. Fever, vomiting, nausea and malaise are common. The episode may resolve though in some cases it may progress to abdominal cramps, tenesmus and frequent passage of small volumes of stool consisting of bloody mucus. Symptoms mostly last for about 4 days, though may continue for 10 days or more (Greenwood et al., 2002).

Three major Shigella species cause disease in man. The most frequently isolated species worldwide is S. flexineriae and accounts for 60% of cases in the developing countries, S. sonnei causes 77% of cases in developed world and 15% of case in developing world while S. dysenteriae type 1 is usually the cause of dysentery epidemics (WHO, 2007). Shigella spp is estimated to cause 164.7 million cases of shigellosis worldwide and 1.1 million deaths (Bardhan et al., 2010).

In Sub-Saharan Africa studies have shown that S. dysenteriae type 1 is associated with bloody diarrhea and dysentery. The organism appeared in Northeast Democratic Republic of Congo in late 1979 and spread to Rwanda and Burundi in 1981, and Tanzania 1982 (Malangreal et al., 1983; Ebright et al., 1984; Mhalu et al., 1984). In 1990 the epidemic spread to Zambia (Tuttle et al., 1996). The epidemics have spread to Southern Africa leading to arise in hospital admissions of children with Shigellosis, and dysentery associated mortality rates (Nathoo et al., 1995). Shigella flexineriae has also been isolated in many regions of Sub-Saharan Africa as a cause of invasive diarrhea (Mikhail et al., 1990). In Kenya, studies have shown that Shigella spp cause
most sporadic bloody diarrhea though *Campylobacter* predominate in children aged 5 years and below (Brook *et al.*, 2003).

### 2.1.3.1 Treatment

*Shigella* species have been shown to be resistant to antibiotics such as ampicillin, chloramphenicol, tetracycline, streptomycin and sulfonamides. Multidrug resistance *Shigella* spp has also been demonstrated in African AIDS patients (Kariuki, 2002). However, antibiotics such as trimethoprim-sulfamethoxazole, norfloxacin, ciprofloxacin or furazolidine may be given when the person is very young or very old, when the disease is severe or when risk of transmission is high. Antidiarrheal drugs (diphenoxylate or loperamide may prolong the infection and should not be used (WHO, 2007).

### 2.1.4 *Campylobacter* species

*Campylobacter* species are Gram negative, microaerophilic, curved or spiral rods bacteria. They have single flagellum at one or both poles which make them to be highly motile. They are transmitted via contaminated food, milk or water and mostly cause sporadic diarrhea in developed world. The most common species are *C. jejuni* and *C. coli*. *Campylobacter jejuni* accounts for 90–95% of infections in most of the world (Greenwood *et al.*, 2002). It has also been detected in higher frequencies in HIVSPPs populations. In South Asia and African continent, *C. jejuni* detection varied between 3.8 to 13.1%, respectively, in diarrheic HIVSPPs (Kownhar *et al.*, 2007). Other spp. namely *C. upsaliensis* and *C. hyointestinalis* cause prolonged mild to moderate diarrhea in HIVSPPs (Jerkin and Tee, 1998). *Campylobacter* present as
dysentery with classic “colitis” symptoms, tenesmus and fever. Lower abdominal pain may be severe, while nausea and vomiting is rare (Wilcox, 2000).

**2.1.4.1 Pathogenesis**

Campylobacteriosis, a gastrointestinal infection caused by *Campylobacter*, is characterized by inflammatory, sometimes bloody diarrhea or dysentery syndrome, mostly including cramps, fever, and pain (Humphrey *et al.*, 2007). The most common routes of transmission are fecal-oral, ingestion of contaminated food or water, and the eating of raw meat. Foods implicated in campylobacteriosis include raw or undercooked poultry, raw dairy products, and contaminated produce (Humphrey *et al.*, 2007). *Campylobacter* is sensitive to the stomach's normal production of hydrochloric acid: as a result, the infectious dose is relatively high, and the bacteria rarely cause illness when a person is exposed to less 10,000 organisms (Vandamme *et al.*, 2006).

The sites of tissue injury include the jejunum, the ileum, and the colon. Most strains of *C. jejuni* produce a toxin that hinders the cells from dividing and activating the immune system. This helps the bacteria to evade the immune system and survive for a limited time in the cells. The organism produces diffuse, bloody, edematous, and exudative enteritis (Jassi*m, 2011).

**2.1.4.2 Treatment**

Standard treatment is now azithromycin, a macrolide antibiotic, especially for *Campylobacter* infections in children although other antibiotics, such as macrolides, quinolones, and tetracycline are sometimes used to treat gastrointestinal *Campylobacter* infections in adults. In case of systemic infections, other bactericidal
antibiotics are used, such as ampicillin, amoxicillin/clavulanic acid, or aminoglycosides. Fluoroquinolone antibiotics, such as ciprofloxacin or levofloxacin, may no longer be effective in some cases due to resistance (Lehtopolku, 2010).

2.1.5 *Clostridium* species

*Clostridium* species are gram-positive, anaerobic and spore-forming rods of the genus *Clostridium*. Diarrhea caused by *Clostridium difficile* is linked to use of broad-spectrum antibiotics such as cephalosporins, clindamycin and quinolones. Pathogenic strains produce enterotoxin (toxin A) and cytotoxin (toxin B), responsible for diarrhea and inflammation in infected patients. It is a common cause of diarrhea in HIV-patients in developed countries; this is likely to be due to the patient’s frequent exposure to antimicrobials and hospitalization which increase colonization (Hutin *et al.*, 1993). In Thailand, Siripan *et al.* (2000) found that infections of *Clostridium difficile* were two times higher in HIV-infected patients than in non-HIV-infected patients. Symptoms of *Clostridium difficile* can appear immediately after beginning of antimicrobial therapy or several weeks after the completion of the therapy. Its clinical presentation is a continuum that includes asymptomatic carriage, diarrhea colitis, pseudo-membranous colitis and fulminate colitis (Wilcox, 2000).

2.1.5.1 Pathogenesis

The use of systemic antibiotics, including any penicillin-based antibiotic such as ampicillin, cephalosporins, and clindamycin, causes the normal bacterial flora of the bowel to be altered. In particular, when the antibiotic kills off other competing bacteria in the intestine, any bacteria remaining will have less competition for space and nutrients. The net effect is to permit more extensive growth than normal of certain
bacteria. *Clostridium difficile* is one such type of bacterium. In addition to proliferating in the bowel, *C. difficile* also produces toxins. Without either toxin A or toxin B, *C. difficile* may colonize the gut, but is unlikely to cause pseudomembranous colitis (Sarah, 2010). The colitis associated with severe infection is part of an inflammatory reaction, with the "pseudomembrane" formed by a viscous collection of inflammatory cells, fibrin, and necrotic cells (Ryan, 2004).

### 2.1.5.2 Prevention

The most effective method for preventing *Clostridium difficile* infection (CDI) is proper antimicrobial prescribing. In the hospital setting, where CDI is most common, nearly all patients who develop CDI are exposed to antimicrobials. Although proper antimicrobial prescribing sounds easy to do, about 50% of antimicrobial use is considered inappropriate. This is consistent whether in the hospital, clinic, community, or academic setting. A decrease in CDI by limiting antibiotics or unnecessary antimicrobial prescriptions reduces CDI (Weiss, 2011).

### 2.1.5.3 Treatment

Carrying *C. difficile* without symptoms is common. Treatment in those without symptoms is controversial. In general, mild cases do not require specific treatment (Nelson, 2011). Oral rehydration therapy is useful in treating dehydration associated with the diarrhea. A number of different antibiotics are used for *C. difficile*, with the available agents being more or less equally effective. Metronidazole is the initial drug of choice for mild to moderate disease. Oral vancomycin is preferred for severe disease (Kelly, 2011). Additionally, vancomycin may be used to treat mild-to-moderate disease if diarrhea persists after a course of metronidazole (Surawicz, 2013).
2.1.6 *Klebsiella* species

The genus *Klebsiella* belongs to the tribe Klebsiellae, a member of the family Enterobacteriaceae. *Klebsiella* are non-motile, rod-shaped, Gram-negative bacteria with a prominent polysaccharide capsule. This capsule account for the largest appearance on samples stained with Gram stain (Cheesbrough, 2000). Serotypes are based on the structural variability of the capsular polysaccharides (K antigens) and lipopolysaccharides (O antigens). There are 77 K antigens and 8 O antigens. The virulence of all serotypes appears to be similar (Cheesbrough, 2000). In recent years *Klebsiellae* have become important pathogens in nosocomial infections. Important manifestations of *Klebsiellae* infection in hospital setting include UTI, pneumonia, bacteremia, wound infection, cholecystitis, and catheter associated bacteriuria. The presence of invasive devices in hospitalized patients greatly increases the likelihood of infection (Ryan and Ray, 2004).

2.1.6.1 Pathogenesis

The bacteria overcome inmate host immunity through several means. They possess a polysaccharide capsule that is the main determinant of their pathogenicity. The capsule is composed of complex acidic polysaccharides. Its massive layer protects the bacterium from phagocytosis by polymorphonuclear granulocytes. In addition, the capsule prevents the bacterial death caused by bactericidal serum factors. The bacteria may also produce multiple adhesins. These help the microorganism adhere to the host cells, which is critical to the infectious process.

Availability of iron increases host susceptibility to *K. pneumonia* infection. Bacteria are able to compete effectively for iron bound to host proteins because of the secretion
of high-affinity, low molecular weight iron chelators known as siderophores. This is necessary because most host iron is bound to intracellular and extracellular proteins. In order to deprive the bacteria of iron, the host also secretes iron-binding proteins.

2.1.6.2 Treatment

*Klebsiella* organisms are resistance to multiple antibiotics (Mandell *et al.*, 2000). This is thought to be a plasmid-mediated property. Length of hospital stay and performance of invasive procedures are risk factors of acquisition of these strains. Treatment depends on the organ system involved. In general, initial therapy of patients with possible bacteremia is empirical. The choice of a specific antimicrobial agent depends on local susceptibility patterns. Once bacteremia is confirmed, treatment may be modified. Agents with high intrinsic activity against *Klebsiella* should be selected for severely ill patients. Examples of such agents include third-generation cephalosporins (cefotaxime, ceftriaxone), aminoglycosides (gentamicin, amikacin) and quinolones. Other antibiotics used to treat susceptible isolates include ampicillin, ceftazidine, cefepime, levofloxacin, norfloxacin, gatifloxacin, moxifloxacin, meropenem and ertapenem (Roberts, 1999).

2.1.7 *Proteus* species

*Proteus* species are part of the Enterobacteriaceae family of Gram-negative bacilli (Braunwald *et al.*, 2001). *Proteus* organisms are implicated as serious cause of infection in humans, along with *Escherichia, Klebsiella, Enterobacter* and *Serratia* species. *Proteus* species are most commonly found in human intestinal tract as part of normal human intestinal flora, along with *Escherichia coli* and *Klebsiella* species, of which *E. coli* is the predominant resident (Mandell *et al.*, 2000).
Proteus is also found in multiple environmental habitats, including long term care facilities and hospitals. *Proteus mirabilis* causes 90% of proteus infections and can be considered a community acquired infection (Braunwald *et al*., 2001). *Proteus mirabilis* remains susceptible to nearly all antimicrobials except tetracycline (Engel and Schaeffer, 1998). Resistance does not appear to be a significant clinical factor, but 10-20% of the strains can acquire resistance to ampicillin and first generation cephalosporins (Bush and Jacoby, 2010).

### 2.1.7.1 Pathogenesis

*Proteus* species possess an extra cytoplasmic outer membrane, a feature shared with other gram-negative bacteria (Mandell *et al*., 2000). In addition, the outer membrane contains a lipid bilayer, lipoproteins, polysaccharides, and lipopolysaccharides. Infection depends on the interaction between the infected organism and the host defense mechanisms. Various components of the membrane interplay with the host to determine virulence. Inoculum size is important and has a positive correlation with the risk of infection. Certain virulence factors have been identified in bacteria. The first step in the infectious process is adherence of the microbe to the host tissue. Fimbriae facilitate adherence and thus enhance the capacity of the organism to produce disease (Roberts, 1999). *Proteus* has fimbriae (pili), which are tiny projections on the surface of the bacterium. Specific chemicals located on the tips of pili enable organisms to attach to selected host tissue sites like urinary tract endothelium. The presence of these fimbriae has been demonstrated to be important for the attachment of *P. mirabilis* to host tissue. The attachment of *Proteus* species to uroepithelial cells initiates several events in the mucosal endothelial cells, including secretion of interleukins 6 and interleukin 8.
Proteus organism also induces apoptosis and epithelial cell desquamation. Bacterial production of urease has also been shown to increase the risk of pyelonephritis in experimental animals. Urease production, together with the presence of bacterial motility and fimbriae, may favor the production of upper urinary tract infections (UTIs) by the organisms such as proteus. Enterobacteriaceae (of which Proteus is a member) and Pseudomonas species are the microorganisms most commonly responsible for Gram-negative bacteremia (Mandell et al., 2000). When these organisms invade the bloodstream, endotoxin, a component of Gram-negative bacterial cell walls apparently triggers a cascade of host inflammatory responses and leads to major detrimental effects. Since Proteus and Pseudomonas organisms are Gram-negative bacilli, they can cause gram-negative endotoxin-induced sepsis, resulting in systemic inflammatory response syndrome (SIRS). SIRS has a mortality rate of 20-50% (Blanco et al., 2008).

2.1.7.2 Treatment and Prevention

Proteus spp infections are treated with broad-spectrum penicillins or cephalosporins except in severe cases. However Proteus spp are not susceptible to nitrofurantoin or tetracycline and have experienced increasing drug resistance of ampicillin, trimethoprim, and ciprofloxin. Proteus mirabilis is part of the normal flora of the gastrointestinal tract, and as a result the bacteria enter the urinary tract or infect medical equipment by the fecal route. Consequently, prevention includes good sanitation and hygiene, including proper sterilization of medical equipment (O’hara et al., 2000).
2.1.8 Pseudomonas aeruginosa

It is a Gram-negative, aerobic, Coccobacillus bacterium with unipolar motility (Ryan, 2004). An opportunistic human pathogen, *P. aeruginosa* is also an opportunistic pathogen of plants (Iglewski, 1996). It is a common bacterium that can cause disease in animals, including humans. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in hypoxic atmospheres, and has, thus, colonized many natural and artificial environments. It uses a wide range of organic material for food; in animals, the versatility enables the organism to infect damaged tissues or those with reduced immunity. *P. aeruginosa* have very simple nutritional requirements (Robin and Janda, 1984). It is often observed, “growing in distilled water” which is evidence of its nutritional needs. In the laboratory, the simplest medium for growth of *P. aeruginosa* consists of acetate for carbon and ammonium sulfate for nitrogen. Its optimum temperature for growth is 37°C and is able to grow at temperatures as high as 42°C. It is resistant to high concentrations of salts and dyes, weak antiseptics and many commonly used antibiotics (Cheesbrough, 2000). These natural properties of the bacterium undoubtedly contribute to its ecological success as an opportunistic pathogen. They also help explain the ubiquitous nature of the organism and its prominence as a nosocomial pathogen (Robin and Janda, 1984).

2.1.8.1 Pathogenesis

The symptoms of such infections are generalized inflammation and sepsis. If such colonization occurs in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal (Balcht and Smith, 1994). Because it thrives on most surfaces, this bacterium is also found on and in medical equipment, including
catheters, causing cross-infections in hospitals and clinics. It is implicated in hot-tub rash. It is also able to decompose hydrocarbons and has been used to break down tar balls and oil from oil spills (Itah, 2005). An opportunistic, nosocomial pathogen of immunocompromised individuals, *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections (Fine, 1996).

### 2.1.8.2 Diagnosis

In diagnosis, a Gram stain is performed, which may show Gram-negative rods and/or white blood cells. *P. aeruginosa* produces colonies with a characteristic 'grape-like' odour on bacteriological media. In mixed cultures, it can be isolated as clear colonies on MacConkey agar (as it does not ferment lactose), which will test positive for oxidase. Confirmatory tests include production of the blue-green pigment pyocyanin on cetrimide agar and growth at 42°C (Hachem, 2007).

### 2.1.8.3 Resistance of *Pseudomonas* to antimicrobial drugs

*P. aeruginosa* is notorious for its resistance to antibiotics and is therefore, a particularly dangerous and dreaded pathogen. The bacterium is naturally resistance to many antibiotics due to the permeability barrier afforded by its outer membrane lipopolysaccharides. Also, its tendency to colonize surfaces in biofilm form makes the cells impervious to therapeutic concentrations of antibiotics. Since its natural habitat is the soil, living in association with the bacilli, actinomycetes and molds, it has developed resistance to a variety of their naturally occurring antibiotics. Moreover, *P. aeruginosa* maintain antibiotics resistance plasmids, both R-factors and RTFs, and it is able to transfer these genes by means of bacterial processes of transduction and conjugation. Only a few antibiotics are effective against *Ps. aeruginosa*, including
fluoroquinolones, gentamicin and imipenem. However even these antibiotics are not effective against all strains. The futility of treatment of *P. aeruginosa* infections with antibiotics is most dramatically illustrated in cystic fibrosis patients, virtually all whom eventually become infected with a strain that is so resistance that it cannot be treated (Bradley, 2002).

2.1.8.4 Treatment

*P. aeruginosa* is frequently resistance to many commonly used antibiotics. Although many strains are susceptible to Gentamicin, to bramycin, colistin, and amikacin, resistant forms have developed. The combination of gentamicin and carbenicillin is frequently used in severe pseudomonas infections. Several types of vaccines are being tested, but none is currently available for general use (Bradley and Jackson, 2014).

2.2 Medicinal plants and traditional medicine

Medicinal plants have been a common source of medicaments, either in the form of crude preparations or as pure active principles (Philipson and Anderson, 1994). The World Health Organization estimate that about 85% of population in sub-Saharan Africa relies on traditional medicine for their primary health care needs (Chooto, 2004). It is estimated that thousands of kilograms of medicinal plants and their parts are collected and used every day by traditional healers and mothers at homes across Africa (Saxena, 2003; Chooto, 2004). Modern analytical methods have revealed the enormous variety and complexity of bioactive principles of medicinal plants but also confirmed their potential value for use as medicines or as model for synthetic drugs (Saxena, 2003).
The use of herbal remedies in the treatment of diseases is universal and traceable to the ancient times when man acquired skills of herbal healing through deliberate selection of plants or by accidental discovery (Wiselogle, 1940; Spencer et al., 1947). Traditional medicine involves the use of herbal medicine, animal parts and minerals. However herbal medicine is the most widely used of the three. Herbal medicines contain active ingredients in, aerial or underground parts of plants or other plant materials, or combination thereof, whether in crude state or as plant preparations (WHO, 2002). The basic principles of the current herbal medicines are a mixture of ancient traditions applied to modern conditions without, in many cases, the benefit of modern science and technology (Verhoef et al., 2005).

In recent years the importance of the utilization of herbal medicine in primary health care system has been emphasized in Africa as well as other countries (Romero-Daza, 2002). In Kenya herbs are traditionally consumed in various ways, most commonly in form of infusion or decoction prepared from the dry plants material. Despite the use of herbal medicine over many centuries, only relatively small number of plant species has been studied for possible medical application. Safety and efficacy data are available for an even smaller number of plants, their extracts active ingredients and preparation containing them (WHO, 2002). The plants used in this study include:

2.2.1 *Boscia angustifolia* (A. Rich.) Capparaceae

This a medium-sized tree 4-6 m high with white bark, growing in semi-arid areas. The branches are very leafy with rather small leaves which are thick, fleshy and hard. Fruit and seeds are edible after cooking. Stripped bark is eaten mixed with millet or as soup. Pieces of boiled wood are used to sweeten milk. The foliage is consumed by
camels and small livestock, especially at the time of flowering and towards the end of the dry season. It is a good source of bee forage (Von, 1986). The tree was formerly converted into charcoal for gunpowder and its hard wood, used in carpentry and water storage vessels. The bark powder is used in ablutions such as application on swollen feet, for kidney pains and stiff neck (Baumer, 1983). The leaves and roots are used to treat diarrhea, pneumonia, boils, chest pain, and wound infections. The fruits are used as a laxative. The vernacular names are Mululue (Kamba), Musambweke (Giriama), Kalkaj (Borana), Mururiatatha (Kikuyu) Bware, Ayiergweng (Luo) (Gacathi, 1989; Beentje, 1994).

2.2.2 Lantana trifolia (L.) Verbenaceae

This species of lantana is an upright tropical, perennial shrub that typically grows to 1.5-2 m tall. Its leaves usually appear in whorls of three. Flower color is typically lavender, but may also be closer to pink or white. Lantanas are useful as honey plants, and are sometimes planted for this purpose, or in butterfly gardening. Decoction of roots and leaves is drunk as a remedy for rheumatism and white growth in the eye (Kokwaro, 1976). Lantana berries are edible when ripe though like many fruit are mildly poisonous if eaten while still green. Extracts of Lantana camara may be used for protection of cabbage against the aphid (Herzog et al., 1996). The vernacular names are Mukenia (Kikuyu), Muvisavisi (Kamba), Kate (Borana), Mvepe (Swahili), Bek-ap-torit (Kipsigis), Lumenenambuli (Luhya), Magwaga (Luo) Ol-makongora (Maasai), Chemosong (Marakwet) and Petiapteriet (Nandi) (Bentjee, 1994).
2.2.3 *Senna spectabilis* (L.) Caesalpiniaceae

This plant species belongs to the family Fabaceae and its common name is yellow shower (English). It is a small, rounded deciduous tree reaching 7-15 m in height and 30 cm in trunk diameter, with a spreading crown. It has yellow flowers and cylindrical brown pods, 30 cm long. It is tolerant to cool conditions and grows in deep, moist, sandy or loamy soils but flourishes even in poor, black cotton soil (Chee, 1984). The tree provides forage for bees, firewood, charcoal and timber for small implements. The timber is heavy, durable, and termite-resistant. Senna leaves and pods are used as safe and effective laxatives. Today, Senna is still recognized as one of the most popular and reliable stimulant laxatives. Some of the vernacular names are Mukengeka, Musingili (Kamba) and Nyai-leka (Luo) (Bentjee, 1994).

2.2.4 *Maytenus putterlickioides* Loes (A. Rich.) Celastraceae

Shrub or small tree 1-3(6) m. high and belongs to the family Celastraceae. It’s often bushy or straggling, with spines up to 1·8 cm. long, terminal or auxiliary on short branches without latex; branches flattened, angular, reddish-brown, reddish-brown- or fawn-pubescent or puberulous when young, and eventually greyish. Leaves are petiolate, elliptic or oblong-elliptic its habitat is dry deciduous woodland, thickets and termite mounds Decoction of the leaves may be mixed with soup and drunk for internal injuries, antiplasmodia and also for cancer or tumors (Kokwaro, 1978).

2.2.5 *Olinia usambarensis* (Gilg.) Oliniaceae

It is also called *Olinia rochetiana* and belongs to the family Oliniaceae. The plant is a shrub or tree 2-12m, bark is grey, smooth or rough and the flowers are cream fading to pink or red. It is commonly found in cedar forest or other types of drier upland
27

forest. *O. usambarensis* is found in diverse mountain forest associations. The tree is distributed throughout East Africa and common in wetter forests. It is found mainly in Kenya and Tanzania and sparsely in Uganda. In Kenya, it occurs on the moist slopes of the Aberdares, Mt Kenya, Taita Hills and Nyambene; it was once a dominant tree in the wet forests of these areas but is now rare everywhere. In Uganda, it occurs in upland and mountain forests, commonly in the Impenetrable (Bwindi), Kalinzu and Kasyoha-Kitomi Forests. In Tanzania, it occurs on Mt Kilimanjaro, the Usambara, Pare and Uluguru Mountains, and in Tukuyu and Iringa (Beentje, 1994). Decoctions of the roots are drunk to cure fever. A decoction from the bark is drunk to cause emission of tapeworms, for rheumatism, bronchitis and indigestion (Kokwaro, 1978). The bark and leaves of *O. usambarensis* are used by traditional herbalists for treating a variety of ailments such as headaches, scabies and madness. It is also used to cure menstrual pain and intestinal worms (Woodcock, 1995). Bark or roots are pounded, water added and the resulting paste applied on swellings such as those on the throat and other tumors. Inner bark may be pounded, mixed with *Brucea* spp and *Myrica salicifolia* and taken in a meat soup as a remedy for abscess, whooping cough and measles. In Kenya, the Taita people boil the bark in water and use it to treat a fatal childhood disease called ‘nyago’ characterized by strong muscular contractions, stomach pains and disturbed breathing, or it may be scraped and the resulting powder used to dress wounds. Malaria and backache are treated using juice obtained from roots that have been pounded and soaked in water (Mbuya et al., 1994).

*O. usambarensis* is a good source of firewood and charcoal. The heartwood is light yellowish-brown, darkening to a deep brown on exposure; sapwood slightly paler, not clearly demarcated. The texture is medium to fine and even; grain interlocked
producing a stripe figure; sometimes lustrous; timber has a distinct camphor scent. The wood seasons well and is resistant to acids and fungi but not to termites. It can be used for furniture, railway-coach frames, joinery, paneling, building poles and the production of veneer (Mbuya et al., 1994). The vernacular names are Mukoda, Muta or Muthangaria (Meru) and Nerkwa (Marakwet) (Kokwaro, 1976).

2.3 Phytochemicals in medicinal plants

Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments (Adebanjo et al., 1983). Proven medicinal plants are used in treatment of diseases either alone or in combination with other plants. They are used as anti-infectious agents, anti-malarial, anti-tumor agents, laxatives, cardiovascular and nerve remedies (Owonubi, 1988). Some biologically active plant-derived secondary metabolites have found application as drugs or as model compounds for drug synthesis and semi-synthesis (Pryde et al., 1981). Some secondary plant metabolites are used though in limited quantities, as pharmacological tools to study various biochemical processes (Baladrin et al., 1985). Diterpene esters from latex of various Euphorbia species are examples of potent irritants and carcinogens, which are useful in studies of chemical carcinogenesis (Pryde et al., 1981).

Traditional medicinal plants have an almost maximum ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Geissman, 1963). Most of these are secondary metabolites, of which 12,000 plant-derived agents have been isolated in the recent past. Many of these substances serve as plant defense mechanisms against invasion by micro-organisms, insects and
herbivores. Some of the plant substances such as terpenoids are responsible for odor (quinones and tannins) plus pigment of the plant (Geissman, 1963). Many compounds are responsible for plant flavor (like the terpenoids capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds (Geissman, 1963). The useful major groups of anti-microbial phytochemicals can be divided into several categories that include alkaloids, flavonoids, quinones, essential oils, lectins, polypeptides, phenolics, polyphenols, tannins and terpenoids (Perumal and Gopalakrishnakone, 2010).

Tannins have been reported to possess cytotoxic and antineoplastic activity (Farnsworth, 1966). Tannins extracted from these trees have been found to inhibit hepatitis “B” virus surface antigen. Tannins have also been reported to prevent development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Fluck, 1973). Flavonoids possess antiviral, anti-inflammatory and cytotoxic activities. Coumarins are known to have anticoagulation, estrogenic, vasodilation, antibacterial, antiviral and antihelmintic properties while anthraquinones like emodins are mainly used as cathartics. In addition, some flavonoids and isoflavonoids isolated from licorice such as licochalcone A, licoisoflavone B, and gancaonal have been reported to exhibit inhibitory activities against *H. Pylori* (Fukai et al., 2002).

Plant steroids have provided valuable medicines for various ailments (Pryde et al., 1981). These include digitalis glycosides (digitoxin or digoxin), which are derived from *Digitalis purpurea*. Digitoxin is cardiotoxic glycosides, which are very effective drugs in hypertension or heart disease management. Alkaloids from *Atropa belladoma*
L. and *Datura stramonium* (L) provide belladonna form which atropine, hyoscymamine and scolamine are synthesized (Farnsworth, 1966). These drugs are used in medicine as parasympatholytic agents, acting in alleviating disorders in central nervous systems (CNS). Saponins are a special class of glycosides which have soapy characteristics and facilitate the reabsorption of food and medicine (Fluck, 1973). It has also been shown that saponins are active antifungal agents (Sodipo et al., 1991).

Drugs discovery from medicinal plants has played an important role in the treatment of cancer and, indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last half century have been applied towards combating cancer (Newman et al., 2003; Butler, 2004). Of all available anticancer drugs between 1940 and 2002, 40% were natural products or natural product-derived with another 8% considered natural product mimics (Newman et al., 2003). Plant derived enzymes of economic importance have also been isolated and purified. Papain and chymopapain, which are derived from *Carica papaya*, L. are best examples and are economically used as meat tenderizers (Pryde et al., 1981).
CHAPTER THREE
MATERIALS AND METHODS

3.1 Collection of medicinal plants

The medicinal plants studied included *Maytenus putterlikioides loes* (A. Rich) Celastraceae, *Boscia angustifolia* (A. Rich) Capparaceae, *Lantana trifolia* (L.) Verbenaceae, *Senna spectabilis* (L.) Caesalpiniaceae and *Olinia usambarensis* (Gilg.) Oliniaceae. They were collected from their natural habitats in Mbeere North District, Embu County, Kenya. A botanist, Mr. Patrick Mutiso from University of Nairobi, School of Biological Sciences assisted in the identification and collection of plant materials with consideration of the bio-conservation aspects.

3.2 Processing and extraction

The plant materials were collected when still fresh and dried under shade at room temperature for 14 days to ensure that they were completely dry and avoid disintegration of active compounds. When completely dry, each medicinal plant sample was ground separately using Laboratory Warring Blender (Clarkson LB10G) for leaves and an electric laboratory mill number 8 for roots and barks. The powdered plant materials were sealed separately in labeled closed plastic containers and were stored at room temperature.

One hundred and twenty five grams of each powdered plant material was later extracted in 1 liter of distilled water at 60°C in a metallic shaker for 6 hours. The mixture was allowed to stand for 6 hours to cool and then decanted into a clean dry conical flask. It was then filtered through folded cotton gauze into another clean dry conical flask. The mixture was then filtered using Whatman No.1 filter paper under
vacuum. The filtrate was then freeze dried in 200ml portions using a Modulyo Freeze Dryer (Edward England) for 48 hours then further dried in a vacuum desiccator over anhydrous copper sulphate. The fine powder obtained was packed in dry plastic bags and stored at room temperature in desiccators.

3.3 Bacterial stocks

Standard microorganisms and clinical isolates were obtained from Kenyatta National Hospital (KNH) and Kenya Medical Research Institute (KEMRI) at the Centre for Microbiology Research (CMR) where they were maintained in a lyophilized state. The organisms included *E. coli*, *S. typhi*, *C. jejuni*, *Shigella dysenteriae*, *Sh. flexineriae*, *Proteus species* and *Pseudomonas aeruginosa*. Standard organisms include *E. coli* ATCC25922, *S. typhi* ATCC19430 and *Pseudomonas aeruginosa* NCTC 10662. Seven diarrhea causing bacteria including known resistant strains of the species were used. In the laboratory, they were sub-cultured in suitable media (Muller Hinton agar) which is suitable for most bacteria, Salmonella Shigella Agar (SSA) for Salmonella and Shigella species and incubated for 24hr at 37ºC. From these sub-cultures, the identities of the organisms was confirmed by colony morphology, gram staining and biochemical tests. The organisms were then sub-cultured into gelatin medium, labeled with code numbers and stored at room temperature.

3.4 Comparative studies

In this study chloramphenicol was used as a standard for comparing the antibacterial potency of the selected medicinal plants extracts. Chloramphenicol was preferred due to its broad spectrum activity against bacteria and also acts as bacteriostatic agent. It is a broad-spectrum antimicrobial agent known to act against chlamydiae, rickettsiae
and most conventional Gram-positive and Gram-negative organisms but *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* are usually resistant (Nguyen *et al*., 2011).

Another choice would have been tetracycline which was originally isolated from a mould called *Streptomyces aureofaciens*. Resistance to tetracycline has, however, become common and the drug is today on use for some specific indications (Patrick *et al*., 2004).

### 3.5 Determination of susceptibility of bacterial isolates to plant extracts

This was carried out using the agar paper disc diffusion method (Barry and Thornberry, 1991; Ojala *et al*., 2000; Rauha *et al*., 2000) which was used to evaluate the antimicrobial activity of plant extracts as a preliminary screening procedure. The media used was Muller Hinton Agar (MHA), also known as Diagnostic Sensitivity Test (DST) Agar. The media was prepared according to the manufacturer’s instructions. The media was then sterilized by autoclaving at 15l Psi pressure (121°C) for 15 min. It was cooled to about 55°C and then poured aseptically onto sterile Petri dishes and left to solidify at room temperature for a few minutes. A 24 hr bacteria cultures, inoculated the previous day and incubated at 37°C was used.

A McFarland standard of 0.5 was prepared by mixing 0.05 mL of 1.175% barium chloride dehydrate (BaCl$_2$•2H$_2$O), with 9.95 mL of 1% sulphuric acid (H$_2$SO$_4$) with constant stirring. This was estimated to give a cell density (1.5x10$^8$ CFU/ml), with optical density (absorbance) of 0.132 at 600nm wavelength. The standard was put in screw cap tubes which were tightly sealed to prevent loss by evaporation. The tubes
were then stored and protected from light at room temperature. The standards were vigorously shaken before use.

A flame sterilized wire loop was used to pick a small part of the 24 hr bacterial colony of each test bacteria and mixed well in sterile 0.89% saline. The turbidity of the bacteria was compared to match the McFarland standard which was assumed to contain \(1.5 \times 10^8\) CFU/ml. Fifty microlitres (50μl) of McFarland standard bacteria was introduced onto each plate. A sterile L-shaped glass rod was used to spread the prepared bacterial culture on to the dry Muller Hinton Agar media and left to dry completely. Using a paper punch whose diameter was 6 mm, paper discs for bioassay were made from Whatman number one filter papers. These discs were then sterilized by autoclaving at 151 Psi pressures (121°C) for 15 minutes in a well-sealed universal bottle.

By this method, one gram of each sample was dissolved in four milliliters of sterile saline and kept as stock solution. The stock solution was sterilized by passing it through 0.45μm pore microfilter. Five microliter containing 1.25 milligram of each stock solution was applied on a sterile paper disk. The paper discs were left to dry at room temperature for a 30 minutes. The extract impregnated paper discs were picked by forceps and placed on to the inoculated DST Agar plates in identified areas. To prevent unacceptable overlapping zones of inhibition, a 90 mm plate accommodated six discs. The saline was included in the assay as negative controls while chloramphenicol served as the positive controls. The inoculated plates with disc were incubated at 37°C for 24 hr.
The diameters of zones (mm) of inhibitions of the test strain were determined with a ruler. Diameters of 6mm (equal to the paper disk) in this study represented no inhibition. Zone size ≤ 9 mm was considered resistant while sizes greater than 9 mm were considered sensitive. Each experiment was repeated five (5) times to ensure that results were reproducible. The extracts that showed highest antibacterial activity, was selected for minimum inhibitory concentration (MIC) and minimum antibacterial concentration (MBC) tests.

3.6 Determination of minimum inhibitory concentration (MIC)

A modified agar micro dilution method (Lorian, 1996) was used to determine the MIC of extracts of the medicinal plants that produces significant inhibition zones with agar paper disc diffusion bioassay method. McFarland standard of each bacteria strain (1.5x10^8 CFU cells/ml) were made as in disc diffusion method. One micro liter of an overnight culture of each bacterial strain was applied onto MHA supplemented with the medicinal plant extracts. Inoculation of bacteria on MHA plates was done. One thousand milligrams (1000 mg) of the extracts were weighed and dissolved completely in 1ml of sterile 0.89% saline to give a concentration of 1000 mg/ml. 1ml of sterile saline was added to end up with 500 mg/ml. Serial double dilutions in saline of 500 mg/ml were made to obtain the following concentrations in milligrams: 500, 250, 125, 62.5, 31.25 and 15.6 mg/ml (Hess et al., 1995; Cheesbrough, 2000). Then 2 ml of each of these concentrations were added to 18 ml of sterilized MHA and cooled to 50°C to make the following final extract concentrations in milligrams per ml: 50, 25, 12.5, 6.25, 3.125 and 1.5 mg. The MHA mixed with extracts on Petri dish plates were left to solidify at room temperature.
Fifty microlitres (50μl) of the McFarland standard of an overnight bacterial culture was inoculated onto MHA supplemented with the medicinal plant extracts. Sterile L-shaped glass rods are then used to spread the prepared bacterial culture on to the dry MHA media and left to dry completely. A control plate to show that bacterial colonies were present and growing accordingly was inoculated and incubated. The plates were incubated at 37ºC for 18-24hr and observations recorded accordingly. The plates showing no bacterial growth were streaked and inoculated on fresh MHA plates without the extracts and incubated for 18hr to confirm that the bacteria were truly inhibited. This procedure was performed repeatedly three times. The results were expressed as the mean values of the lowest concentration of plant extracts that produced complete suppression of colony growth which was taken as the MIC. An antimicrobial agent with low activity against an organism has a high MIC while a highly active antimicrobial agent gives a low MIC.

3.7 Determination of Minimum bactericidal concentration (MBC)

The broth dilution MBC method was used to quantitatively measure the in vitro activity of an antimicrobial agent against a bacterial isolate. The minimum bactericidal concentrations (MBC) of plant extracts bioassays were done according to the methods by Boaky-Yiadon (1979). Briefly, 1000mg of extracts were weighed and completely dissolved in 1ml of sterile 0.89% saline to give a concentration of 1000mg/ml. Double serial dilutions of the extracts were made in 0.89% saline to give the following concentrations in mg/ml: 500, 250, 125, 62.5, 31.25 and 15.6. A ten times dilution was made of the extracts by adding 0.2 mL of the extract into 1.8mls of Muller Hinton Broth (MHB) to make the following final concentrations in milligrams/mL: 50, 25, 12.5, 6.25, 3.125 and 1.5 (Ayofor et al., 1994; Hess et al., 1995).
Nine tubes for each test organism were set at every concentration. A McFarland standard of each bacterial strain of $1.5 \times 10^8$ CFU was made, and then 50$\mu\ell$ McFarland standard of each test organism was added to different extract concentrations. The tubes were then incubated at 37°C for 18hr. At the end of incubation period, the tubes were examined for turbidity to determine whether there was bacterial growth.

To confirm growth, 50$\mu\ell$ from each tube was collected and plated onto nutrient agar and incubated at 37°C for 18hr. Nutrient agar with no bacterial growth indicated negative score, confirming that the extracts had actually killed the bacteria at that concentration, while plates with bacterial growth were taken as positive, confirming that the extracts had merely inhibited bacteria at that concentration. The lowest concentration of the plant extracts that did not yield any colony growth in Muller Hinton Broth (MHB) after the incubation period was taken as the MBC. The minimum bactericidal concentration was read as the lowest extract concentration which yielded no bacterial growth upon subculture.

This procedure was repeated three times and results expressed as the mean values of the lowest concentration of plant extracts that were completely bactericidal to the bacteria colony growth. Antimicrobial agents with low activity against an organism had a high MBC while a highly active antimicrobial agent gave a low MBC.

3.8 In vivo toxicity testing

3.8.1 Experimental animals

Twenty (20) male Swiss albino mice (3-4 weeks old) that weighed an average of 18 g were used in the study. They were bred in the animal house at the Département of
Biochemistry and Biotechnology of Kenyatta University. The mice were housed at a temperature of 25°C with 12h/12h darkness-light photoperiod cycles and fed on rodent pellets and water ad libitum. The mice were divided into four different groups of five mice each. One group served as the untreated control. The other three groups were treated with 1g/kg body weight of the extracts. The three extracts that were active against the diarrhea causing pathogenic bacteria in previous bioassay were subjected to in vivo toxicity tests. The three extracts were administered orally to each of the three groups accordingly on daily basis for a period of a month. Saline was administered to the fourth group as negative control. During this period, the mice were fed ad libitum to mice pellets and allowed free access to water. They were observed for any signs of general illness, change in behavior and mortality. At the end of one month the mice were sacrificed.

The body weight of each mouse was taken using an electronic beam balance model type BL-220H, (SHIMADZU CORPORATION JAPAN) during the acclimatization period, before and after commencement of dosing, once weekly during the dosing period and on the day of sacrifice. On the day of sacrifice, the animals were euthanized by use of chloroform and later sacrificed (Henry et al., 1960). Different organs namely the heart, liver, lungs, spleen, kidneys, intestines and testicles were carefully dissected out and weighed in grams. Necropsy samples were collected and stored in 10% formalin. The tissues were processed using the standard protocols of histopathology.
3.8.2 Histopathology

The formalin fixed organs (heart, liver, lungs, spleen, kidneys, intestines and testicles) were trimmed to include all major parts to be investigated and washed in running water overnight to remove excess formalin. The tissues were then processed using an automatic tissue processor (Leico Histokinette 2000). The tissues were dehydrated sequentially in increasing concentrations (50, 70, 90, and absolute) of alcohol. Tissues were washed for 2hr in 100% alcohol. The tissues were then cleared of alcohol twice in two changes of Xylene. Infiltration was done by passing them through molten paraffin wax for 6hrs, 3hr in each wax bath. The tissues were embedded in fresh molten wax in embedding moulds. Embedded tissues were then attached to wooden block and sectioned at 0.5 μm thicknesses with a microtome and floated in warm water bath at 54°C to spread out, then attached onto a clean microscopic slide. After holding in hot oven for 15 minutes, the tissue sections were dewaxed in Xylene and then stained with haematoxylin and eosin dyes using standard histological protocols. The stained tissues were cover slipped with DPX mountant, dried and examined microscopically for any pathological changes.

3.8.3 Biochemical assays

At the end of the experimental period (4 weeks), all animals were exsanguinated and 4 ml of blood samples were drawn from the heart of each sacrificed animal. The samples were collected in plastic test tubes without an anticoagulant and allowed to stand for 3hr to ensure complete clotting. The clotted blood samples were centrifuged at 3000 rpm for 10min and clear serum samples were aspirated off and stored frozen at -20°C. Serum sample analysis was done using the Olympus 640 Chemistry Auto Analyzer (AU 640 OLYMPUS). This is a discrete, random access clinical analyzer.
capable of performing a wide range of chemistry tests in a single run. All reagents for the machine were commercially obtained to fit the required volumes and concentration. The reagents were in specific containers referred to as "reagent cartridges". The reagent cartridges were bar coded for the identification by the machine.

The machine was programmed for the selected tests for each sample. The sample sectors were then placed into the autoloader assembly. A number of events that occurred simultaneously were performed automatically under direct control of the instrument microprocessors. The analyzer was able to determine the activity of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Blood Levels of Urea (BUN), Creatine Kinase (CK), Gamma-glutamyltransferase (γ-GT), Glucose (Glu), Uric acid (UA), Lactate dehydrogenase (LDH) and Urea compared with the quality control runs.

3.8.4 Determination of Hematological Parameters

Blood was examined using standard protocols (Jain, 1986). Red blood cells, white blood cells, hemoglobin and mean hematocrit count were determined using the Coulter Count System Beckman Coulter® (Thermo Fisher, UK). The machine automatically dilutes a whole-blood sample of 30µℓ, lyses and counts and gives a print-out result of absolute numbers of white blood cells (WBC) (expressed as number of cells x 10⁹ per liter) and red blood cells (RBC) number of cells x 10¹² per liter). In addition Hb (in grams per liter) and MCHC (in grams per deciliter) were measured by the analyzer. The autoanalysers utilizes two independent methods. These methods are the independent method for determining the white blood cells (Coulter, 1956), and the
modified cyanomethemoglobin method for determining the hemoglobin (International Committee for Standardization in Haematology, 1978).

Differential leucocytes counts (DLC) for neutrophils, lymphocytes, basophils, eosinophils and monocytes were determined from stained blood films (Jain, 1986). Air-dried thin blood films stained with giemsa stain were examined microscopically using magnification 400 and 1000 for differential WBC counts and cell morphologies respectively. Neutrophil (N), lymphocyte (L) and monocytes (M) absolute counts (number of cells x 10^9) per liter were obtained by expressing their percent differential counts against the total WBC absolute counts (Jain, 1986).

3.9 Qualitative Phytochemical Screening

Phytochemical screening for major constituents was undertaken using standard qualitative methods. The freeze dried extracts were tested for alkaloids, tannins, steroids, triterpenes, flavonoids and anthraquinones according to the method of Harborne (1973) and Sofowora (1993). The presence or absence of these secondary metabolites was confirmed by color changes of solutions after the addition of the testing reagents. Results were read and indicated as (+ve), showing the presence and (–ve) showing the absence of the phytochemicals.

3.9.1 Test for alkaloids

The crude extract (2g) was hydrolyzed with 2 ml of 2% HCl solution by heating in a water bath for 10 min., then a few drops of Mayer’s reagent was added to 5 ml of the filtrate. The appearance of turbidity indicated the presence of alkaloids (Harborne, 1973; Trease and Evans, 1978).
3.9.2 Test for tannins

The test for tannins was carried out by dissolving 3g of each plant extract in 6ml of distilled water. This was filtered and then 1ml of ferric chloride reagent added to it. The occurrence of a blackish blue color showed the presence of gall tannins and a green blackish color indicated catechol tannins (Harborne, 1973; Sofowora, 1993).

3.9.3 Test for anthraquinones

A few drops of 0.5 M KOH was added to 1 ml of the extract followed by 1ml of 3% hydrogen peroxide. The mixture was boiled for a few min and then cooled and filtered. Five milliliter of the solution was made acidic by adding acetic acid and then extracted with benzene. To the benzene layer, ammonium solution was added. Appearance of a reddish color indicated the presence of anthraquinones.

3.9.4 Test for triterpenes and steroids

A crude (2 g) extract was defatted with n-hexane and the residue extracted with 40ml of chloroform. To 0.5 ml portion of the chloroform extract, 0.5ml of acetic acid was added followed by 2 drops of concentrated sulphuric acid. Gradual color change to green-blue confirmed the presence of sterols and triterpenes (Wall, 1952; Sofowora, 1993).

3.9.5 Test for saponins

Saponins have the ability to produce frothing in aqueous solution and to hemolyse red blood cells; the property which was used as screening test for the compounds. The extract was subjected to frothing test for the identification of saponin. Hemolysis test
was further performed on the frothed extracts in water to remove false positive results (Wall, 1952; Sofowora, 1993).

3.9.6 Test for flavonoids

A crude (1g) extract was defatted by extracting with n-hexane. The residue was dissolved in 80% ethanol (30 ml) and treated as follows: (a) 4ml of 1% aluminum chloride in methanol solution was added to 3 ml of the ethanol solution. The appearance of a pale yellow color indicated the possible presence of flavonoids. (b) Ethanol solution (2ml) was reacted with 0.5 ml of concentrated hydrochloric acid and a few magnesium turnings. Red color appearance indicated the presence of flavonoids (Kapoor, 1969; Earnsworth et al., 1974).

3.9.7 Test for coumarins

Coumarins were detected in plant material simply by placing a small amount of moistened sample in a test tube and covering the tube with filter paper moistened with dilute sodium hydroxide solution. The covered test tube was then placed in a boiling water bath for several minutes, and then the paper was removed and exposed to ultraviolet light. The presence of coumarins was detected by a yellow-green fluorescence appearance within a few minutes (Franklin et al., 1961).

3.9.8 Test for reducing sugars

One milliliter (1ml) of the aqueous extract was diluted with 2 ml of water, 1 ml of Felling’s solution was added and the mixture heated. A brick-red precipitate indicated the presence of reducing sugar.
3.10 Determination of mineral element content

This was carried out by use of Total X-ray Fluorescence Spectroscopy (TXRF) which is a versatile analytical technique for determining elemental content in liquids, solids and loose powders. The main principle is that atoms, when irradiated with X-rays, radiate secondary X-rays known as the fluorescence radiation. Each element is associated with a specific wavelength and energy of the fluorescence radiation. The concentration of each element is calculated using the fluorescence intensity. This spectroscopy combines high accuracy and precision with simple and fast sample preparation for the analysis of elements from Sodium (Na; 11) to Uranium (U; 92) in concentration ranges from 100% to sub-ppm level is a powerful diagnostic tool for agriculture and environment.

A primary beam generated by an X-ray tube is monochromatized by Bragg-reflection on a Ni/C multilayer. Monochromatized X-rays hit a polished sample carrier (quartz glass or acrylic glass) at a very shallow angle of incidence. X-rays are totally reflected by the surface of the sample carrier at a very small angle (0.3 – 0.6°). Fluorescence radiation is emitted only by the sample deposited on the carrier surface. The characteristic fluorescence radiation emitted by the sample is detected by an energy-dispersive detector. Intensity is measured by means of an amplifier coupled to a multichannel analyzer. Sample digestion is not normally required. Samples are prepared on a sample tray that reflects X-ray radiation. Trays have a diameter of 30 mm, made of acrylic or quartz glass. Liquids are prepared directly on the sample tray. Several µl are transferred to the glass disc using a pipette and evaporated. Powdered samples are finely ground (<50µm), prepared in suspensions and transferred to the
sample tray. Powder samples (a few µg of sample) can also be transferred directly, using a Q-tip, for semi-quantitative analysis.

### 3.11 Data management and statistical analysis

The results were represented in form of Tables and Figures. The data collected was entered into Ms Excel speed sheets where it was organized for statistical analysis. Analysis of data was done using SPSS statistical software version 20. The antimicrobial activity of the selected medicinal plant extracts was initially tested by Disk Diffusion method. All isolates with zone of inhibition equal or greater than 9 mm were regarded as susceptible to the extracts hence the extracts were selected for quantitative test. For the MIC and MBC values, a regression analysis was used to analyze mean MIC and MBC for each of the selected organisms. Student T test was used to evaluate safety of the plant extracts by comparing the means of untreated group of normal mice (control) with group treated with the extracts. Data was expressed as mean ± SD. The value P<0.05 was considered statistically significant.
CHAPTER FOUR

RESULTS

4.1 Preliminary Screening of Plant Extracts

4.1.1 Yield of plant extracts

Upon extraction and freeze drying *Maytenus putterlickiioides loes* (roots) had the highest yield compared with the rest of the extracts while *Lantana trifolia* (roots) had the lowest after freeze drying (Table 4.1).

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Extract in g per 100g of plant material</th>
<th>Color of freeze dried plant extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Boscia angustifolia</em> (Leaves)</td>
<td>5.6</td>
<td>Redish brown</td>
</tr>
<tr>
<td><em>Boscia angustifolia</em> (Roots)</td>
<td>6.44</td>
<td>Light red brown</td>
</tr>
<tr>
<td><em>Maytenus putterlickiioides</em></td>
<td>8.16</td>
<td>Light brown</td>
</tr>
<tr>
<td>loes (aerial)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Maytenus putterlickiioides</em></td>
<td>7.8</td>
<td>Redish brown</td>
</tr>
<tr>
<td>loes (Stem)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Maytenus putterlickiioides</em></td>
<td>9.6</td>
<td>Brownish</td>
</tr>
<tr>
<td>loes (Roots)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lantana trifolia</em> (Leaves)</td>
<td>5.88</td>
<td>Black granules</td>
</tr>
<tr>
<td><em>Lantana trifolia</em> (Roots)</td>
<td>5.12</td>
<td>Dark brown granules</td>
</tr>
<tr>
<td><em>Senna spectabilis</em> (Leaves)</td>
<td>6.6</td>
<td>Black paste</td>
</tr>
<tr>
<td><em>Olinia usambarensis</em> (Leaves)</td>
<td>8.52</td>
<td>Dark brown granules</td>
</tr>
<tr>
<td><em>Olinia usambarensis</em> (Roots)</td>
<td>8.92</td>
<td>Light brown granules</td>
</tr>
</tbody>
</table>

4.1.2 Antibiotics susceptibility testing

The antibiotics used in this study inhibited the growth of bacterial isolates over a diameter of 10 to 22mm as shown in table 4.2. Ampicillin produced the lowest zone of inhibition to isolated microorganisms. Chloramphenicol had the most inhibitory antibiotic effect with inhibitory zones ranging from 10 to 22 mm. The results also show that most of the bacterial isolates were resistance to ofloxacin, ampicillin, and tetracycline, amikacin (Table 4.2).
Tukey’s multiple comparison showed that the effect of chloramphenicol was similar for *S. flexineri*, *P. aeruginosa* and *P. vulgaris* while it was different for *S. typhi*, *E. coli* and *C. jejuni* (*p < 0.05*). Ofloxacin induced a similar effect on *S. typhi*, *E. coli* and *S. dysenteriae* and were significantly different from *S. flexineriae*, *P. aeruginosa* and *P. vulgaris* (*p < 0.05*). On the other hand, ampicillin produced a similar effect on *S. typhi*, *E. coli*, *P. aeruginosa* and *C. jejuni* (*p > 0.05*). However, they were significantly different from *S. dysenteriae*, *S. flexineriae*, and *P. vulgaris* (*p < 0.05*). Moreover, tetracycline caused a similar effect on *S. typhi*, *E. coli*, *S. flexineriae*, *P. aeruginosa* and *P. vulgaris* (*p > 0.05*). However, these were significantly different for its effects on *S. dysenteriae* and *C. jejuni* (*p < 0.05*). Nalidixic produced a similar effect on two different groups of bacteria (*E. coli*, *P. aeruginosa*, *P. vulgaris*, *C. jejuni*) and (*S. dysenteriae*, *S. flexineri*) (*p > 0.05*). However, each group was significantly different from the other (*p < 0.05*). Moreover, each individual bacterial effect was significantly higher in *S. typhi* Compared to all the other types of bacteria (*p < 0.05*).

A similar effect was indicated on the effect on amoxicillin on the different species of bacteria. There was a similar effect on two different groups of bacteria (*E. coli*, *S. dysenteriae*, *P. aeruginosa*, *C. jejuni*) and (*S. flexineri*, *P. vulgaris*) (*p > 0.05*). However, each group was significantly different from the other (*p < 0.05*). Moreover, each individual bacterial effect was significantly higher in *S. typhi* compared to all the other types of bacteria (*p < 0.05*). The effect of Amikacin was similar on all the types of bacteria (*p >0.05*) except on its effect on *E. coli*. 

The effect of Ciprofloxacin was also similar on all the bacteria except for *S. dysenteriae, S. flexineri* which were observed to have significantly higher disk values (p < 0.05). An almost similar effect was also observed for the effect of cefotaxime on the different bacterial isolates except that

A similar effect of Ceftazidime was observed with similar diffusion disk values for *S. dysenteriae, S. flexineriae, P. aeruginosa, P. vulgaris* and *C. jejuni* (p > 0.05). This species bacterial isolates were significantly different from *S. typhi* and *E. coli* (p < 0.05). A similar effect of Cefotaxime was observed just as in ceftazidime except that only *E. coli* and *C. jejuni* was significantly different from *S. typhi, S. dysenteriae, S. flexineri, P. aeruginosa, P. vulgaris*. 
Table 4.2: Zone of inhibition (millimeters) of respective antibiotics against bacterial isolates

<table>
<thead>
<tr>
<th></th>
<th><em>S. typhi</em></th>
<th><em>E. coli</em></th>
<th><em>S. dysenteriae</em></th>
<th><em>S. flexineri</em></th>
<th><em>P. aeruginosa</em></th>
<th><em>P. vulgaris</em></th>
<th><em>C. jejuni</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHL</strong></td>
<td>11.7±2.3&lt;sup&gt;bg&lt;/sup&gt;</td>
<td>21.7±3.1&lt;sup&gt;acef&lt;/sup&gt;</td>
<td>10.0±1.0&lt;sup&gt;abef&lt;/sup&gt;</td>
<td>16.0±1.0&lt;sup&gt;cb&lt;/sup&gt;</td>
<td>15.7±0.6&lt;sup&gt;cb&lt;/sup&gt;</td>
<td>15.0±1.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>17.0±1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>OLFL</strong></td>
<td>6.0±0.0&lt;sup&gt;def&lt;/sup&gt;</td>
<td>6.0±0.0&lt;sup&gt;def&lt;/sup&gt;</td>
<td>6.0±0.0&lt;sup&gt;def&lt;/sup&gt;</td>
<td>14.3±0.6&lt;sup&gt;acg&lt;/sup&gt;</td>
<td>13.3±0.6&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>15.0±1.7&lt;sup&gt;abcdg&lt;/sup&gt;</td>
<td>6.7±0.6&lt;sup&gt;def&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>AMP</strong></td>
<td>6.0±0.0&lt;sup&gt;cdfg&lt;/sup&gt;</td>
<td>7.0±0.8&lt;sup&gt;cdfg&lt;/sup&gt;</td>
<td>12.3±0.6&lt;sup&gt;abde&lt;/sup&gt;</td>
<td>17.0±0.6&lt;sup&gt;abceg&lt;/sup&gt;</td>
<td>6.3±0.6&lt;sup&gt;acdf&lt;/sup&gt;</td>
<td>14.0±2.1&lt;sup&gt;beg&lt;/sup&gt;</td>
<td>6.0±0.0&lt;sup&gt;cdf&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>TETRA</strong></td>
<td>6.0±0.0&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>6.0±0.0&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>7.7±0.6&lt;sup&gt;abefg&lt;/sup&gt;</td>
<td>6±0.6&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>6.0±0.0&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>6.0±0.6&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>14.3±1.2&lt;sup&gt;acdef&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>NAL</strong></td>
<td>19.3±0.6&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>6.3±0.6&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>12.3±0.6&lt;sup&gt;abefg&lt;/sup&gt;</td>
<td>13.0±0.6&lt;sup&gt;abefg&lt;/sup&gt;</td>
<td>6.0±0.0&lt;sup&gt;efcd&lt;/sup&gt;</td>
<td>6.3±0.6&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>6.3±0.6&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>AMOX</strong></td>
<td>14.3±0.6&lt;sup&gt;bcfg&lt;/sup&gt;</td>
<td>6.7±1.2&lt;sup&gt;af&lt;/sup&gt;</td>
<td>7.7±0.6&lt;sup&gt;af&lt;/sup&gt;</td>
<td>11.3±2.3&lt;sup&gt;abceg&lt;/sup&gt;</td>
<td>8.0±1.0&lt;sup&gt;af&lt;/sup&gt;</td>
<td>11.3±0.6&lt;sup&gt;bcfg&lt;/sup&gt;</td>
<td>6.0±0.0&lt;sup&gt;af&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>AMIK</strong></td>
<td>6.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.3±1.5&lt;sup&gt;acfg&lt;/sup&gt;</td>
<td>6.7±0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.3±0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.7±0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>CIPRO</strong></td>
<td>12.3±2.5&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>12.0±1.0&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>19.0±1.0&lt;sup&gt;abefg&lt;/sup&gt;</td>
<td>22.6±2.3&lt;sup&gt;abefg&lt;/sup&gt;</td>
<td>13.0±2.0&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>9.3±0.6&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>13.7±1.5&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>CEFT</strong></td>
<td>13.7±0.6&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>17.0±1.0&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>6.3±0.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.7±1.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.0±0.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.3±0.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.7±1.2&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>CEFO</strong></td>
<td>6.0±0.0&lt;sup&gt;bg&lt;/sup&gt;</td>
<td>14.7±0.6&lt;sup&gt;acdefg&lt;/sup&gt;</td>
<td>6.0±0.0&lt;sup&gt;bg&lt;/sup&gt;</td>
<td>7.7±0.6&lt;sup&gt;bg&lt;/sup&gt;</td>
<td>7.3±0.6&lt;sup&gt;bg&lt;/sup&gt;</td>
<td>7.0±1.0&lt;sup&gt;bg&lt;/sup&gt;</td>
<td>12.0±1.0&lt;sup&gt;abdef&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as means ±SD of three determinations. Diameter of inhibition zone (DIZ) of 6mm shows no inhibition, DIZ<9 shows resistant and DIZ≥9 shows sensitive. Values marked with letters as a superscript indicates groups with which they are significantly different. Letters that are similar suggests no significance difference.

Key: CHL-Chloramphenicol; OLF-Olfloxacin; CIPR-Ciprofloxacin; CEFT-Ceftazidime; AMP-Ampicillin; AMOX-Amoxicillin; TET-Tetracycline; AMIK-Amikacin; NAL-Nalidixic acid; CEFO-Cefotaxime.
4.1.3 Susceptibility of bacterial isolates to plant extracts

Table 4.3 shows the effect of plant extracts against bacterial isolates. *Boscia angustifolia* (Leaves), *Maytenus putterlickioides* (stem), *Boscia angustifolia* (roots), *Lantana trifolia* (roots) and *Olinia usambarensis* (roots) showed inhibition zones ranging from 6.0-7.8mm. *Lantana trifolia* (leaves) and *Maytenus putterlickioides* aerial parts) were slightly active against *Sh. flexineri* with zone of inhibition ranging 7.9-8.1mm. *Maytenus putterlickioides* (roots) and *Senna spectabilis* (leaves) were active against *Sal. typhi*, *Sh. flexineri* and *Sh. dysenteriae* with zone of inhibition ranging 9.2-15.8mm. *Olinia usambarensis* (leaves) had antimicrobial activity against several bacterial isolates with zone of inhibition ranging 9-15mm. The most sensitive organism was *Shigella flexineri* and the least was *Pseudomonas aeruginosa*.

Tukey’s multiple comparison showed that M1 caused a similar effect on *E. coli, S. dysenteriae, P. aeruginosa, P. vulgaris and C. jejuni* (p >0.05). This effects were significantly different from those induced on *S. typhi* and *S. flexineriae* (p <0.05). On the other hand, M2 produced a significantly different diffusion diffusion disk values between *E. coli* and *S. flexineri* (p < 0.05). Similarly, the disk diffusion value for *S. flexineri* was significantly different from *S. typhi, E. coli, P. aeruginosa and C. jejuni* (p < 0.05). M3 caused a significant inhibition for *S. flexineri when compared to all the other groups* (p < 0.05). However, all the other groups were basically similar in the disk diffusion values. No significant variations were observed for the disk diffusion values for the different bacterial isolates for M4. Only *S. flexineri* was significantly different from all the other bacterial isolates with the use of M5. Use of M6 produced a similar effect in *P. aeruginosa and C jejuni* (p >0.05) when compared to *dysenteriae, P. aeruginosa* (p < 0.05). Moreover, M6 has a similar effect on *S. typhi and P. vulgaris*
when compared to *S. flexineri* (*p < 0.05*). However, individually, the extracts of M6 generated a significant varied effect on *E. coli*, *S. dysenteriae* and *S. flexineri* (*p < 0.05*). Extract M7 was also found to induce a significant inhibition of growth on several bacterial isolates. *E. coli* and *C. jejuni* had a similar significant and comparable effect to on *S. typhi*, *S. flexineri* and *P. vulgaris* (*p <0.05*). Similarly, on *S. typhi* and *P. vulgaris* and *S. dysenteriae* had all significantly different disc diffusion values from *E. coli*, *S. flexineri* and *C. jejuni* except for *S. typhi* which was also significantly different to *P. aeruginosa* while *S. dysenteriae* was significantly different from *S. typhi*, *P. aeruginosa* and *P. vulgaris* (*p < 0.05*). Both M8 and M9 did not produce any inhibition zones when subjected to the different bacterial isolates.

The extract M10 also induced a similar effect on *S. typhi*, *E. coli*, *S. dysenteriae* and *S. flexineri* which had significantly different disc diffusion disc values when compared to *P. aeruginosa*, *P. vulgaris* and *C. jejuni* (*p < 0.05*). In addition, *S. typhi* was also significantly different from *S. dysenteriae* and vice versa. Use of M10 caused a similar variation in the disc values of *P. aeruginosa* and *P. vulgaris*. 
Table 4.3: Zone of inhibition (millimeters) of aqueous extracts of selected plants against bacterial isolates

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>S. typhi</th>
<th>E. coli</th>
<th>S. dysenteriae</th>
<th>S. flexineri</th>
<th>P. aeruginosa</th>
<th>P. vulgaris</th>
<th>C. jejuni</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
</tr>
<tr>
<td>M1</td>
<td>7.4±1.1bdefg</td>
<td>6.0±0.0^d</td>
<td>6.2±0.5^ad</td>
<td>7.2±0.8abefg</td>
<td>6.0±0.0^ad</td>
<td>6.0±0.0^ad</td>
<td>6.0±0.0</td>
</tr>
<tr>
<td>M2</td>
<td>6.0±0.0</td>
<td>6.2±0.5^d</td>
<td>7.6±0.6</td>
<td>7.8±0.8^abeg</td>
<td>6.8±1.1</td>
<td>7.4±1.7</td>
<td>6.4±0.6</td>
</tr>
<tr>
<td>M3</td>
<td>6.4±0.6^d</td>
<td>6.0±0.0^d</td>
<td>7.2±1.3^d</td>
<td>7.8±0.8^abefg</td>
<td>6.0±0.0^d</td>
<td>6.0±0.0^d</td>
<td>6.0±0.0</td>
</tr>
<tr>
<td>M4</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
</tr>
<tr>
<td>M5</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
<td>7.4±0.6^bcfg</td>
<td>6.0±0.0</td>
<td>6.0±0.0</td>
</tr>
<tr>
<td>M6</td>
<td>9.2±1.1^d</td>
<td>9.6±0.6^defg</td>
<td>11.0±1.0^bg</td>
<td>12.2±1.9^abfg</td>
<td>8.0±0.7^cd</td>
<td>9.8±1.3^d</td>
<td>7.0±0.7^cd</td>
</tr>
<tr>
<td>M7</td>
<td>9.6±0.6^bdeg</td>
<td>6.0±0.0^adff</td>
<td>12.8±0.8^abhcfg</td>
<td>15.8±1.6^abefg</td>
<td>7.6±0.5^ad</td>
<td>9.6±1.1^bdg</td>
<td>6.0±0.0^af</td>
</tr>
<tr>
<td>M8</td>
<td>7.8±1.8</td>
<td>7.2±1.3</td>
<td>6.4±0.6</td>
<td>7.9±1.5</td>
<td>6.0±0.0</td>
<td>6.4±0.6</td>
<td>6.0±0.0</td>
</tr>
<tr>
<td>M9</td>
<td>6.8±1.1</td>
<td>6.0±0.0</td>
<td>7.0±0.7</td>
<td>8.1±1.3</td>
<td>6.0±0.0</td>
<td>6.8±0.5</td>
<td>6.0±0.0</td>
</tr>
<tr>
<td>M10</td>
<td>12.2±0.8^cdefg</td>
<td>14.2±1.6^defg</td>
<td>15.0±1.0^aefg</td>
<td>13.8±2.1^efg</td>
<td>9.2±1.3^abcd</td>
<td>9.0±0.7^abcd</td>
<td>9.6±0.9^abd</td>
</tr>
<tr>
<td>M11</td>
<td>11.7±2.3</td>
<td>21.7±3.1</td>
<td>10.0±1.0</td>
<td>16.0±1.0</td>
<td>15.7±0.6</td>
<td>15.0±1.0</td>
<td>17.0±1.0</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ±SD of five determinations; (DIZ) of 6mm shows no inhibition, DIZ<9 shows resistant and DIZ≥9 shows sensitive. Values marked with letters as a superscript indicates groups with which they are significantly different. Letters that are similar suggests no significance difference. Analysis was determined by ANOVA followed by tukey’s post hoc analysis.

**Keys:** M1 – *Boscia angustifolia* (Leaves); M2 – *Maytenus putterickioides* (stem); M3 – *Olinia usambarensis* (roots); M4 – *Lantana trifolia* (roots); M5 – *Boscia angustifolia* (roots); M6 – *Maytenus putterickioides* (roots); M7 – *Senna spectabilis* (leaves); M8 – *Lantana trifolia* (Leaves); M9 – *Maytenus putterickioides* (aerial parts); M10 – *Olinia usambarensis* (Leaves); M0-0.89% Saline; M11-Chloramphenical
4.2 Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of *S. spectabilis*, *M. putterlickioides*, and *O. usambarensis* aqueous extracts

Table 4.4 shows results of activity of three aqueous plant extracts tested against the various strains of bacterial isolates. The aqueous extract of *S. spectabilis* produced a MIC range of 12.5 to 50 mg/ml and MBC range of 25 to 75 mg/ml for all bacterial species except *C. Jejuni* and *Ps. aeruginosa*. The aqueous extract of *M. putterlickioides* aqueous produced a MIC range of 6.25-50 mg/ml and MBC range of 12.5-75 mg/ml against the various strains of bacterial isolates except *C. jejuni*. The aqueous extract of *O. usambarensis* gave a MIC range of 6.25 to 50 mg/ml and MBC range of 12.5-75 mg/ml for all bacterial isolates (Table 4.4).
Table 4.4: Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of the plant extracts.

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Chloramphenicol</th>
<th>M. Putterlickioides (Roots)</th>
<th>S. spectabilis (Leaves)</th>
<th>O. usambarensis (Leaves)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (mg/L)</td>
<td>MBC (mg/mL)</td>
<td>MIC (mg/mL)</td>
<td>MBC (mg/mL)</td>
</tr>
<tr>
<td>Sal. typhi</td>
<td>0.063±0.01</td>
<td>0.143±0.02</td>
<td>52±2.74</td>
<td>73±2.74</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.063±0.01</td>
<td>0.113±0.01</td>
<td>52±2.74</td>
<td>73±2.22</td>
</tr>
<tr>
<td>Sh. dysenteriae</td>
<td>0.063±0.01</td>
<td>0.25±0.01</td>
<td>25±1.81</td>
<td>37±1.14</td>
</tr>
<tr>
<td>Sh. flexineri</td>
<td>0.063±0.01</td>
<td>0.25±0.01</td>
<td>12.5±0.82</td>
<td>25.5±2.12</td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>1.2±0.05</td>
<td>4.8±0.20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pr. vulgaris</td>
<td>3.9±0.05</td>
<td>22.8±0.64</td>
<td>50±2.72</td>
<td>75±2.73</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>0.75±0.03</td>
<td>5.0±0.01</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SD of five determinations. Differences between means of the MICs and MBCs were analyzed using students’ test of (SPSS version 20 program instat).
4.3 Phytochemical composition of selected plant extracts

The selected plant extracts contained alkaloids, tannins, anthocyanins, triterpenes and steroids, saponins, flavanoids, coumarins and reducing sugars in varying quantities. Tannins, alkaloids, saponins and flavanoids were most abundant in *O. usambarensis* leaves, while *S. spectabilis* leaves were rich in both coumarins and also alkaloids. *Maytenus putterlickioides* show high presence of tannins and saponins but low level of alkaloids. Reducing sugars were only found in trace quantities in all plant samples (Table 4.5).

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th><em>Senna spectabilis</em> (Leaves)</th>
<th><em>Maytenus putterlickioides</em> (Roots)</th>
<th><em>Olinia usambarensis</em> (Leaves)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenes &amp; steroids</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Coumarins</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Reducing Sugars</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: +++ the reaction is strongly positive; ++ reaction is moderately positive; + reaction is slightly positive; - reaction is negative

4.4 Mineral concentration in µg/g in the plant extracts, mineral elements intake in µg/day/mouse and Recommended Daily allowance µg/day/mouse

Table 4.6 shows the amount of minerals present in the medicinal plants extracts and the amount orally administered to mice (average weight of 18g) at 1g/kg body weight dose per day (µg/day) for thirty days. Results shows that the daily administration of aqueous plants extracts provided minerals such as K, Ca, Mn, Fe, Cu, Ti, Ni and Zn at levels below the recommended daily requirements. The rest of the minerals in the medical plant extracts that could cause toxicity were also at negligible levels.
<table>
<thead>
<tr>
<th>Mineral Element</th>
<th>S. spectabilis Conc. µg/g</th>
<th>Daily intake µg/day</th>
<th>M. putterlickioides Conc. µg/g</th>
<th>Daily intake µg/day</th>
<th>O. usambarensis Conc. µg/g</th>
<th>Daily intake µg/day</th>
<th>RDA for mouse/day (µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>15400 ± 92</td>
<td>277.20</td>
<td>19879 ± 91</td>
<td>358</td>
<td>12667 ± 362</td>
<td>228</td>
<td>4.25x10⁴</td>
</tr>
<tr>
<td>Ca</td>
<td>27331 ± 112</td>
<td>491</td>
<td>1334 ±9</td>
<td>24</td>
<td>2681 ± 34</td>
<td>48</td>
<td>6.15x10⁴</td>
</tr>
<tr>
<td>Ti</td>
<td>47.9 ± 0.7</td>
<td>0.9</td>
<td>9.30 ± 1.28</td>
<td>0.17</td>
<td>35.2 ± 0.2</td>
<td>0.63</td>
<td>1.5*</td>
</tr>
<tr>
<td>V</td>
<td>13.2 ± 2.3</td>
<td>0.24</td>
<td>2.83 ± 0.24</td>
<td>0.05</td>
<td>11.9 ± 1.4</td>
<td>0.21</td>
<td>0.31</td>
</tr>
<tr>
<td>Cr</td>
<td>&lt;0.2</td>
<td>&lt; 0.0036</td>
<td>&lt;0.2</td>
<td>&lt; 0.0036</td>
<td>&lt;0.2</td>
<td>&lt; 0.0036</td>
<td>0.010</td>
</tr>
<tr>
<td>Mn</td>
<td>220 ± 12</td>
<td>3.96</td>
<td>59.9 ± 1.0</td>
<td>1.08</td>
<td>108 ± 6</td>
<td>1.94</td>
<td>520</td>
</tr>
<tr>
<td>Fe</td>
<td>338 ± 4</td>
<td>6.08</td>
<td>120 ± 4</td>
<td>2.16</td>
<td>221 ± 5</td>
<td>3.98</td>
<td>1.27x10³</td>
</tr>
<tr>
<td>Co</td>
<td>&lt;0.1</td>
<td>&lt; 0.002</td>
<td>1.06 ± 0.38</td>
<td>&lt;0.02</td>
<td>0.513 ± 0.010</td>
<td>&lt;0.009</td>
<td>0.20</td>
</tr>
<tr>
<td>Ni</td>
<td>1.33 ± 0.32</td>
<td>0.024</td>
<td>10.9 ± 0.9</td>
<td>0.20</td>
<td>5.20 ± 0.37</td>
<td>0.09</td>
<td>0.28*</td>
</tr>
<tr>
<td>Cu</td>
<td>2.47 ± 0.07</td>
<td>0.44</td>
<td>11.3 ± 0.3</td>
<td>0.20</td>
<td>6.15 ± 0.09</td>
<td>0.11</td>
<td>0.32*</td>
</tr>
<tr>
<td>Zn</td>
<td>19.4 ± 0.6</td>
<td>0.35</td>
<td>11.2 ± 0.2</td>
<td>0.20</td>
<td>21.2 ± 1.2</td>
<td>0.38</td>
<td>6.79</td>
</tr>
<tr>
<td>As</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Br</td>
<td>26.0 ± 1.0</td>
<td>0.45</td>
<td>81.8 ± 1.0</td>
<td>1.47</td>
<td>26.1 ± 1.1</td>
<td>0.47</td>
<td>8.0x10⁴</td>
</tr>
<tr>
<td>Rb</td>
<td>14.4 ± 0.1</td>
<td>0.26</td>
<td>63.8 ± 1.4</td>
<td>1.15</td>
<td>30.2 ± 0.9</td>
<td>0.54</td>
<td>0.5</td>
</tr>
<tr>
<td>Sr</td>
<td>169 ± 0.5</td>
<td>3.04</td>
<td>34.3 ± 0.6</td>
<td>0.62</td>
<td>47.9 ± 1.1</td>
<td>0.86</td>
<td>None</td>
</tr>
<tr>
<td>Y</td>
<td>2.64 ± 0.94</td>
<td>0.048</td>
<td>4.17 ± 1.51</td>
<td>0.075</td>
<td>4.36 ± 1.33</td>
<td>0.078</td>
<td>None</td>
</tr>
<tr>
<td>Hg</td>
<td>&lt;0.05</td>
<td>0.0009</td>
<td>&lt;0.05</td>
<td>0.0009</td>
<td>&lt;0.05</td>
<td>0.0009</td>
<td>None</td>
</tr>
<tr>
<td>Pb</td>
<td>2.43 ± 0.09</td>
<td>0.044</td>
<td>0.594 ± 0.028</td>
<td>0.011</td>
<td>2.54 ± 0.08</td>
<td>0.046</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Results for mineral concentrations are expressed as Mean±SD for three replicates for each plant extract while results for Daily mineral administered are expressed as µg per day of medical plants extracts orally administered to mice of average weight of 18g. The values with asterisk indicate the upper limit of the mineral element.
4.5 In vivo Toxicity Studies

4.5.1 Effects of oral administration of 1g /kg bw of plant extract on weekly weights of mice
Results show that oral administration of 1g/kg body weight dose of *M. putterlickioides* increased the weight gain significantly (p <0.05) in the third and fourth week relative to day zero (Table 4.7). The average change in weekly weight showed a significant increase of weight following the administration of 1g/kg body weight dose of *M. putterlickioides* in mice (p <0.05). There was a steady decline in weight for *O. usambarensis* at 1g/kg body weight dose in mice for the second, third and fourth week relative to the control mice (p <0.05). The average change in weekly weight showed a significant loss of weight following the administration of 1g/kg body weight dose of *S. spectabilis* and *O. usambarensis* in mice (p <0.05).

Table 4.7: Effects of oral administration of aqueous plant extracts at 1g/kg body weight daily for 28 days on weekly body weight and average weekly change in weight in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weekly Weight of mice (g)</th>
<th>ΔWeight /Week)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>17.0±2.0</td>
<td>18.6±2.2</td>
</tr>
<tr>
<td><em>S. spectabilis</em></td>
<td>17.6±1.5</td>
<td>18.1±2.13</td>
</tr>
<tr>
<td><em>M. putterlickioides</em></td>
<td>17.8±1.1</td>
<td>18.7±0.7</td>
</tr>
<tr>
<td><em>O. usambarensis</em></td>
<td>20.7±0.8*</td>
<td>19.5±1.1</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SD for five mice per group. Difference between mean weekly change in body weight of the control mice and mice treated with each of the plant extracts was compared using the student’s test. Values labeled with a superscript (*) suggest variation in significant change in weekly weight. Values marked with an (**) suggests significant change in weight within the week. Significant variations are reported at ρ < 0.05.

4.6 Effects of oral administration of aqueous plant extracts at 1g/kg body weight daily for 28 days on the percent relative organ to body weights in mice

Table 4.8 shows the effect of oral administration of aqueous extracts of *S. spectabilis, M. putterlickioides* and *O. usambarensis* to mice at 1g/kg body weight daily for 28
days on percent relative organ to body weights. Results show that \textit{S. Spectabilis} produced an insignificant elevation of the relative percent organ to body weight of all the body organs. There was high of the heart and testis elevated following the administration of \textit{M. puterlickioides} while the rest of the organs had marginal elevation. However, this was highly elevated with the administration of extracts from \textit{O. usambarensis} for all the body organs. Tukey’s multiple comparison showed that administration of \textit{O. usambarensis} for the 28 days caused a significant increase in the weights of the liver, brain, kidney, lungs, spleen and heart (p < 0.05).
Table 4.8: Effects of oral administration of aqueous plant extracts at 1g/kg body weight daily for 28 days on the percent relative organ to body weights in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver</th>
<th>Brain</th>
<th>Kidney</th>
<th>Lung</th>
<th>Spleen</th>
<th>Testis</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.27±0.64</td>
<td>1.10±0.07</td>
<td>1.02±0.07</td>
<td>0.96±0.13</td>
<td>0.81±0.07</td>
<td>0.58±0.02</td>
<td>0.30±0.05</td>
</tr>
<tr>
<td><em>S. spectabilis</em></td>
<td>7.82±2.65*</td>
<td>1.50±0.49*</td>
<td>1.59±0.45*</td>
<td>1.34±0.50*</td>
<td>1.24±0.52*</td>
<td>0.71±0.09*</td>
<td>0.64±0.20*</td>
</tr>
<tr>
<td><em>M. putterlickioides</em></td>
<td>6.24±0.46</td>
<td>1.15±0.22</td>
<td>1.38±0.19</td>
<td>0.98±0.21</td>
<td>0.91±0.11</td>
<td>0.86±0.13*</td>
<td>0.51±0.08*</td>
</tr>
<tr>
<td><em>O. usambarensis</em></td>
<td>8.24±0.48*</td>
<td>1.62±0.13*</td>
<td>1.73±0.13*</td>
<td>1.40±0.09*</td>
<td>1.39±0.14*</td>
<td>0.66±0.03</td>
<td>0.61±0.06*</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SD for five mice per group. Differences between relative organ weighs of mice treated with each of the plant extracts and the control mice was compared using the students’ test *p<0.05 was considered statistically significant.
4.7 Effect of oral administration of 1g/kg body weight of plant extracts on hematological parameters in mice

Oral administration of *O. usambarensis* aqueous extract to mice at 1g/kg body for 28 days caused a decrease in PCV, MCV, PCT and MPV significantly (p < 0.05). On the other hand, the MCHC was significantly elevated (p < 0.05). The effect of *M. putterlickioides* extracts caused decrease in the PCT significantly compared with the control (p < 0.05).

### Table 4.9: Effects of oral administration of aqueous plant extracts at 1g/kg body weight daily for 28 days on hematological parameters in mice

<table>
<thead>
<tr>
<th>Hematological Parameters</th>
<th>Control</th>
<th><em>S. spectabilis</em></th>
<th><em>M. putterlickioides</em></th>
<th><em>O. usambarensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (x10³/µL)</td>
<td>11.28±1.18</td>
<td>8.725±1.44</td>
<td>9.5±1.88</td>
<td>11.98±1.70</td>
</tr>
<tr>
<td>RBC (x10⁶/µL)</td>
<td>8.23±1.08</td>
<td>8.05±0.99</td>
<td>8.91±0.9</td>
<td>7.25±0.10</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>11.34±0.60</td>
<td>11.05±0.34</td>
<td>12.3±1.28</td>
<td>9.86±1.42</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>58.7±3.10</td>
<td>57.19±3.92</td>
<td>63.36±34</td>
<td>34.18±4.19*</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>72.0±6.13</td>
<td>69.13±1.29</td>
<td>71.24±47</td>
<td>47.4±4.08*</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>13.96±1.6</td>
<td>13.88±1.44</td>
<td>13.82±0.92</td>
<td>13.66±1.50</td>
</tr>
<tr>
<td>PLT (x10³/µL)</td>
<td>799.8±127</td>
<td>863±79</td>
<td>954.4±158</td>
<td>613±215</td>
</tr>
<tr>
<td>Ly (%)</td>
<td>81.94±3.09</td>
<td>83.05±1.76</td>
<td>85.54±3.98</td>
<td>79.96±0.98</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>17.14±1.69</td>
<td>18.25±2.1</td>
<td>18.32±2.77</td>
<td>16.42±1.51</td>
</tr>
<tr>
<td>PCT (%)</td>
<td>0.41±0.03</td>
<td>0.45±0.04</td>
<td>0.57±0.16*</td>
<td>0.18±0.06*</td>
</tr>
<tr>
<td>MPV (fL)</td>
<td>5.64±0.91</td>
<td>5.25±0.24</td>
<td>4.64±2.58</td>
<td>3.22±0.62*</td>
</tr>
<tr>
<td>PDW (%)</td>
<td>18.06±0.61</td>
<td>18.28±0.72</td>
<td>17.83±1.26</td>
<td>17.84±0.43</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SD for five animals per group. Difference between mean hematological parameters of the control mice and mice treated with each of the plant extracts are compared using the students t test *P<0.05 was considered statistically significant.

4.8 Effects of oral administration of aqueous plant extracts at 1g/kg body weight daily for 28 days on differential white blood cell count (DLC) in mice

Table 4.10 shows the effect of oral administration of aqueous extracts of *S. spectabilis, M. putterlickioides* and *O. usambarensis* to mice at 1g/kg body weight daily for 28 days on some differential white blood cell count (DLC) in mice. The
results show that oral administration of aqueous extracts of *M. putterlickioides* significantly decreased the monocytes count compared to those in the control mice. However, a similar dose of *O. usambarensis* significantly decreased the neutrophils and increased the lymphocytes count compared to those in the control mice (p <0.05). *S. spectabilis* did not have any effect on the differential counts of the white blood cells when compared to the control (p > 0.05).
Table 4.10: Effects of oral administration of aqueous plant extracts of 1g/kg body weight daily for 28 days on differential white blood cell count in mice

<table>
<thead>
<tr>
<th>Treatment (mg/kg/day)</th>
<th>Differential white blood cell count</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neutrophils (%)</td>
<td>Lymphocytes (%)</td>
</tr>
<tr>
<td>Control</td>
<td>30.2±1.48</td>
<td>51.4±1.14</td>
</tr>
<tr>
<td><em>S. spectabilis</em></td>
<td>28±1.83</td>
<td>54±1.83</td>
</tr>
<tr>
<td><em>M. putterliokiodis</em></td>
<td>28.2±0.84</td>
<td>54.8±1.64</td>
</tr>
<tr>
<td><em>O. usambarensis</em></td>
<td>27.2±0.84*</td>
<td>55.6±1.14*</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± SD for five mice per group. Differences between Differential leucocytes count (DLC) of mice treated with each of the plant extracts and the control mice was compared using the students’ test *p<0.05 was considered statistically significant.
4.9 Effect of oral administration of aqueous plant extracts at 1g/kg body weight daily for 28 days on biochemical parameters in mice

Table 4.11 shows the effect of oral administration of aqueous plant extracts at 1g/kg body weight daily for 28 days on some end point biochemical parameters in mice. Results shows that oral administration of aqueous extracts of *S. spectabilis* at 1g/kg body weight daily for 28 days significantly increased the levels of blood uric acid while *O. usambarensis* extracts at the same dose and time significantly increased the activity of aspartate aminotransferase (AST) activity and AST/ALT ratio (p <0.05). *M. putterlickioides* did not significantly influence the liver and kidney enzyme activities (p <0.05).
Table 4.11: Effects of oral administration of aqueous plant extracts 1g/kg body weight daily for 28 days on biochemical parameters in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>UA (µM)</th>
<th>LDH (U/L)</th>
<th>CK (U/L)</th>
<th>AST/ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54.4±17.0</td>
<td>266.2±34</td>
<td>21.6±1.8</td>
<td>151.8±20</td>
<td>3999±203</td>
<td>621.6±21.3</td>
<td>5.4±1.8</td>
</tr>
<tr>
<td>S spectabilis</td>
<td>35.5±17.0</td>
<td>215±34</td>
<td>4.5±1.8</td>
<td>283±20*</td>
<td>3844±203</td>
<td>321±21.3</td>
<td>7.6±1.8</td>
</tr>
<tr>
<td>M putterlikioides</td>
<td>36.0±4.1</td>
<td>244±94</td>
<td>3.8±0.7</td>
<td>138±74</td>
<td>3539±247</td>
<td>652±99</td>
<td>7.0±3.6</td>
</tr>
<tr>
<td>O usambarensis</td>
<td>45.0±9.7</td>
<td>542±20.7*</td>
<td>9.4±2.1</td>
<td>174±61</td>
<td>4576±474</td>
<td>578±63</td>
<td>11.0±3.5*</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SD for five animals per group. Difference between mean of the measured biochemical parameters of the control mice and mice treated with each of the plant extracts was compared using the student’s t test. *P<0.05 was considered statistically significant.
4.10 Histopathology

Oral administration of aqueous plant extracts at 1g/kg body weight daily for 28 days in mice did not cause any histopathological change of heart, lungs, spleen and testis (data not shown) relative to the control mice. However, oral administration of aqueous plant extracts of *S. spectabilis*, *M. putterlickioides* and *O. usambarensis* at 1g/kg body weight daily for 28 days in mice caused histopathological changes in the liver, kidney and intestine specimens compared to the control mice (Plates 1-12).

4.10.1 The Liver

The observed normal liver of mouse treated with normal saline for 28 days shows intact hepatocytes, clear veins and sinusoid as show with the arrow in (Plate 1). Liver specimen from mice treated orally with *M. putterlickioides* and *S. spectabilis* at (1g/kgbw/day) for 28 days show intact hepatocytes, normal uncongested central veins and sinusoids (Plate 3 and Plate 4, respectively). Liver specimen from mice treated orally with *Olinia usambarensis* (1g/kgbw/day) for 28 day revealed liver infiltration and venous congestion (Arrow, Plate 2).

Plate 1: Photomicrograph of a histological section of a liver of a mouse orally treated with normal saline, for 28 days. Note: intact hepatocytes, normal uncongested central veins and sinusoids Haematoxylin and eosin stain Mg X 400.
Plate 2: Photomicrograph of a histological section of a liver of a mouse orally treated with an aqueous extract of *Olinia usambarensis* (1g/kgbw/day) for 28 days Note: intact hepatocytes, congested central veins and sinusoids (Arrow). Haematoxylin and eosin stain Mg X 400.

Plate 3: Photomicrograph of a histological section of a liver of a mouse orally treated with an aqueous extract of *M. putterlickioides* (1g/kgbw/day) for 28 days Note: intact hepatocytes, normal uncongested central veins and sinusoids. Haematoxylin and eosin stain Mg x 400.

Plate 4: Photo-micrograph of a histological section of a liver of a mouse orally treated with an aqueous extract of *Senna spectabilis* (1g/kgbw/day) for 28 days Note: intact hepatocytes, normal uncongested central veins and sinusoids. Haematoxylin and eosin stain Mg x 400.
4.10.2 Kidney

Kidney of mice orally treated with normal saline and those treated orally with an aqueous extract of *M. putterlickioides* (1g/kgbw/day) for 28 days show normal epithelium and glomerulus (Plate 5 and 6, respectively). However kidney of mice orally treated with an aqueous extract of *Senna spectabilis* and *O. usambarensis* (1g/kgbw/day) for 28 days show intense infiltration of inflammatory cells causing necrosis and loss of cellular details (Arrow Plate 7 and 8).

**Plate 5**: Photo-micrograph of a histological section of a kidney of a mouse orally treated with normal saline, food and water for 28 days. Note: Normal epithelium and glomerulus. Haematoxylin and eosin stain. Mg x 400.

**Plate 6**: Photomicrograph of a histological section of a kidney of a mouse orally treated with an aqueous extract of *M. putterlickioides* (1g/kgbw/day) for 28 days. Note: Normal epithelium and glomerulus. Haematoxylin and eosin stain Mg x 400.
Plate 7: Photo-micrograph of a histological section of a kidney of a mouse orally treated with an aqueous extract of *Senna spectabilis* (1g/kgbw/day) for 28 days Note: There intense infiltration of inflammatory cells causing necrosis and loss of cellular details (Arrow). Haematoxylin and eosin stain Mg x 400.

Plate 8: Photomicrograph of a histological section of a kidney of a mouse orally treated with an aqueous extract of *O. usambarensis* (1g/kgbw/day) for 28 days Note: There intense infiltration of inflammatory cells causing necrosis and loss of cellular details (Arrow) Haematoxylin and eosin stain Mg x 400.

4.10.3 Intestine

Intestine of mice orally treated with normal saline, and *M. putterlichioides* (1g/kgbw/day) for 28 days showed the normal structure of villi (well aligned with cells) (Arrow in plates 9 and 10, respectively). Intestines of mice orally treated with an aqueous extract of *Senna spectabilis* and *O. usambarensis* (1g/kgbw/day) for 28 days revealed villi disintegration and lack of continuity of the villi coating (Arrow in Plate 11 and 12, respectively).
Plate 9: Photomicrograph of a histological section of intestine of a mouse orally treated with normal saline, food and water for 30 days. Note: the normal structure of villi (well aligned with cells) (Arrow). Haematoxylin and eosin stain. Mg x 400.

Plate 10: Photomicrograph of a histological section of intestine of a mouse orally treated with an aqueous extract of *M. puterlickioides* (1g/kgbw/day) for 28 days Note: the normal structure of villi (well aligned with cells) (Arrow). Haematoxylin and eosin stain Mg x 400.

Plate 11: Photo-micrograph of a histological section of intestines of a mouse orally treated with an aqueous extract of *Senna spectabilis* (1g/kgbw/day) for 28 days. Note: Villi disintegration and lack of continuity of the villi coating (Arrow). Haematoxylin and eosin stain Mg x 400.
Plate 12: Photo-micrograph of a histological section of intestines of a mouse orally treated with an aqueous extract of *O. usambarensis* (1g/kgbw/day) for 28 days. Note: Villi disintegration and lack of continuity of the villi coating (Arrow). Haematoxylin and eosin stain Mg x 400.
CHAPTER FIVE
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION
The global perspective in the treatment and control of diseases is slowly changing from the use of conventional drugs to herbal applications due to preference of organic products but also due to their easier accessibility and affordability. This is particularly so in the sub-Saharan Africa where the World Health Organization (WHO) estimates that, 85% of the population relies on herbs for their primary health care needs (Chooto, 2004). There is lack of comprehensive laboratory data to support claims and little information on their toxicity and no credibility on their use.

In this study, 100 g of each dried plant materials yielded a range of 5.12 to 9.6 g of fine freeze-dried extract. The study was carried out to evaluate five selected plants for their antibacterial activity against human bacterial pathogen that cause diarrhea. This was accomplished by determining the susceptibility of the organisms to the extracts, its potency and compared with chloramphenicol, a conventional antibiotic. Chloramphenicol was used in the study as it is a known antibacterial agent with broad spectrum of activity and also due to its bacteriostatic mode of action (William et al., 1965).

Antibiotics inhibited the growth of bacterial isolates over the range 6mm to 22mm. Ampicillin produced the lowest zone of inhibition to microorganism's isolates while chloramphenicol was the most inhibitory antibiotic with inhibitory zones ranging from 10mm to 22mm. This agrees with other previous studies reported by Skinner and Ahmad (1994). The result also shows that most of the bacterial isolates were resistant
to ampicillin, tetracycline and amikacin. In terms of sensitivity to antibiotics, *Shigella dysenteriae* were the most sensitive bacteria isolates while *Pseudomonas aeruginosa* was the least sensitive. This agrees with previous studies reported by Sawer et al. (1995). The findings reveal that most of the bacterial isolates are sensitive to common antibiotics like chloramphenicol and fairly sensitive to cephalosporin.

Three out of the ten selected medicinal plants extracts tested by disk diffusion technique had inhibitory activity on most bacterial isolates with inhibition diameter ranging from 9mm to 18mm against 6mm for the negative control. These are *Senna spectabilis* (Leaves), *Maytenus putterlikioides* (Roots) and *Olinia usambarensis* (leaves). This inhibitory activity could be contributed by the phytochemicals present in these plant extracts. The plant extracts contain alkaloids, tannins, saponins, flavonoids, anthocyanins, triterpenoids, coumarins and reducing sugars in varying amounts. Alkaloids such as ramiflorines A and B, crytolepine, and quinolones have been reported to be active against both gram positive and gram negative bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Shigella flexineri* and *Shigella boydii* (Karou et al., 2005; Tanaka et al., 2006).

Flavonoids are reported to be active against both gram positive and gram negative bacteria such as *Bacillus Subtilis*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Akroum et al., 2009). Coumarins such as cniforin A and edulin are reported to be active against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Ng et al., 1996). Saponin extracts have been reported to be active against *Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Proteus mirabilis*.
(Abbasolu and Turkoz, 1995). Crude tannin fraction has been reported to inhibit the growth of *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* (Doss et al., 2009). Camaric acid is active against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* (Innocent, 2011).

The MICs for selected organisms ranged from 3.2-50mg/ml and MBC from 6.25-75mg/ml. The MIC:MBC ratio of chloramphenicol to the selected bacterial isolates is 1:2. The significant difference in the mean MIC values for chloramphenicol and the plant extracts implies that chloramphenicol has significantly higher potency than the plant extracts. However, considering that chloramphenicol is a pure compound and the plant extracts are crude products, then it is prudent to infer that the plant extracts have considerable effect against bacteria. This implies that in pure form, the plant extracts may be more potent against some species of bacteria that cause diarrhea than the crude extract. If a plant extract exhibit similar values for MIC and MBC against the test bacteria, this shows that the MIC of the extract indicates the bactericidal activity while if the extract exhibits an MBC greater than MIC, then the MIC of the extract indicates a bacteriostatic activity. The MBC of the plant extracts was higher than MIC; this is similar to Chloramphenicol thus suggesting the mode of action may be closely related. This shows that the plant extracts are bacteriostatic in nature because they could not eliminate bacteria even at a higher MBC values as determined by microtitre method.

Bacteriostatic agents have low potency but increase in their concentrations can increase their potency. Low concentrations of bactericidal agents are known to be
bacteriostatic (Bernard et al., 1980) and the agents act mainly by inhibiting cell wall, nucleic acid and protein synthesis or inhibition of transcription (Leonard et al., 1988). The long-term survival of bacterial strains in the presence of antibiotic even beyond 24 hours is due to the production of osmotically stable L-forms that remain viable, multiply and grow to vegetative forms after the antibiotic is inactivated. The two types of L-forms include unstable L-form which can divide and revert to the original morphology while stable L-forms are unable to revert to original forms. L forms of bacteria are strains of bacteria that lack cell walls (Leaver et al., 2009). Since bacterial isolates survived even after 24 hours in this study, it shows they are the stable L-form. The L forms are generated in a culture medium that has the same osmolarity as the bacterial cytosol, which prevents cell lysis by osmotic shock (Allan, 1991). Pseudomonas aeruginosa is very adaptable and tolerant to antibiotics (Morse et al., 1986). The resistance attributed to Ps. aeruginosa could be capsule related (Woolfrey et al., 1990).

Bacteriostatic agents are more preferred in treatment of infections other than those requiring emergency attention because they allow the body to develop acquired immunity against the pathogens by delaying the elimination but suppressing multiplication of the same thereby giving the body a chance to fight the agents through the immune mechanism (Lawrence et al., 1997). Therefore, the phytochemicals present in these plant extracts contributed to their antibacterial activity against diarrhea causing bacteria pathogen and thus justifies their continued use in traditional medicine.
The toxicity of the active plants extracts against the bacterial isolates was assessed to ascertain whether they are suitable for therapeutic purposes without causing toxicity to the host cells. The white blood cell differential count is often used as part of a complete blood count (CBC) as a general health check. It may be used to help diagnose the cause of a high or low white blood cell (WBC) count, as determined in a CBC. It may also be used to help diagnose and/or monitor other diseases and conditions that affect one or more different types of WBCs. The low level of neutrophils, eosinophils and monocytes in mice treated with of *S. spectabilis* and *M. putterlickioides* at a rate of 1g per kg body weight/day for 28 days, compared to the control mice shows that the mice reacted to the plant extract. The reduction in these types of white blood cells, when plant extracts of *S. spectabilis* and *M. putterlickioides* were administered orally, may imply a reduction in the ability of the body to respond to infection (Kaushansky, 1995). This can be explained by the abnormal increase in size of the spleen thus affecting the lymphoid follicles which contain B lymphocytes and lymphoid populations. Lymphoid depopulation/lymphoid follicle reduction could be caused by the presence of cytotoxic saponins which destroy the lymphocytes. Similar toxicity pattern was observed from histopathological changes due to saponin in mice (Diwan *et al.*, 2000).

The significant increase in lymphocytes observed on oral administration of plant extracts of *O. usambarensis* is associated with a more accelerated production of these cells and a boosted immunity to mice by these extracts (Kaushansky, 1995). The increased lymphocytes (main effectors cells of the immune system) indicate a possible stimulatory effect by these extract on lymphocytes production (McKnight *et al.*, 1999). This could be due to tissue damage caused by some constituents of the
plants extracts. This argument is in line with the observed enlargement of the liver, brain, kidney, lung, spleen and heart of mice orally administered with 1g *O. usambarensis* extracts/kg body weight per day for 28 days. The fact that oral administration of *O. usambarensis* extract did not increase white blood cell count might imply that biotransformation of this extract components may be essential for this effect to be expressed. This shows that *O. usambarensis* extract does not have advance negative effects on the immune system of the mice as compared to the other extract.

Body weight maintenance can be achieved through manipulation of energy expenditure (EE), mainly heat production also known as thermogenesis, appetite suppression/satiety enhancement, fat and glucose absorption blocking. Either one or more components can be altered by the phytochemicals. Most phytochemicals that affect body weight regulation have a complex mechanism of action, where their main effect is to increase or decrease body weight. The site of main mechanism of action includes central nervous system (CNS), peripheral or both.

Some free fatty acids (FFA) significantly increase the release of satiety hormones such as cholecystokinin (CCK) (Pasman *et al.*, 2008). CCK delays gastric emptying and produces a subsequent increased feeling of satiety and a decreased appetite. In terms of inducing satiety-hormone secretion, long chain fatty acids are more effective than medium chain fatty acids. Also polyunsaturated fatty acids (PUFAs) are more effective than monounsaturated fatty acids (MUFAs) (Lawton *et al.*, 2000).
Alkaloids such as $\rho$-octopamine and synephrines exert adrenergic agonist activity aid weight loss. Synephrines potentially increase energy expenditure (EE) and decrease food intake. In addition, there is some evidence that adrenergic agonists, decrease gastric motility (Astrup, 2000). Alkaloid test was positive in all the plant extracts but was strongly positive in *O. usambarensis* which show a remarkable decrease of the mice body weight after administration of 1g of plant extract per kg body/day daily for 28 days.

Flavone glycosides, saponins and various other flavonoids have appetite suppressant action that has peripheral and central effects. In the adipose tissue, glycosides reduce lipogenesis (Preuss *et al.*, 2004). In the central structures regulating appetite, glycosides and its related molecules have mechanism where they act by amplifying the signaling of the energy sensing function in the hypothalamus (Kuriyan *et al.*, 2007). The reduced growth rate in animals treated with *O. usambarensis* extract relative to that of control may also be due to tannins. Tannins reduce feed intake by decreasing palatability and reducing feed digestion. Tannins reduce palatability because they are astringent. Astringency is the sensation caused by the formation of complexes between tannins and salivary glycoprotein. Low palatability results in reduced feed intake hence poor performance. Reduced digestibility influence feed intake negatively because of the filling effect associated with undigested feedstuff.

Tannins are classified into hydrolysable and condensed tannins. Hydrolysable tannins are converted by microbial metabolism and gastric digestion into absorbable low molecular weight metabolites such as tannic acid which are toxic. The major lesions associated with hydrolysable tannins poisoning are hemorrhagic gastroenteritis which
decreases absorption of nutrients, necrosis of the liver and kidney damage with proximal tubular necrosis. Hemorrhagic gastroenteritis was confirmed histologically by the infiltration of villus stroma by more inflammatory cells and minimal oedema of the intestines of animal administered with 1g of *O. usambarensis* extract /kg body weight. Villi disintegration was also observed and there was no continuity of the villi coating.

The condensed tannins like Protanthocyanidins (PAs) retard growth by inhibiting feed intake and digestibility (Click, 1969). Protanthocyanidins which are not absorbed by the digestive tract, damage the mucosa of the gastrointestinal tract, decreasing the absorption of nutrients such as proteins, carbohydrates and essential amino acids such as methionine and lysine. They also increase excretion of proteins and essential amino acids as well as altering the excretion of certain cations. This reduced body weight in extract treated animals could also be partly explained by the increase in weight of the kidney, spleen and heart in animals treated with *S. spectabilis* and *M. putterlickioides*. Animals treated with *O. usambarensis* had an increase in weight of all the examined organs except the testis.

Organ weights are widely accepted in the evaluation of samples associated with toxicity (Black, 2002; Wooley, 2003). Organ weight changes are often associated with treatment related effects. The STP recommends that liver, heart, kidneys, brain, adrenal glands, and testes (preferably from sexually mature animals) should be weighed in all species in multidose general toxicology studies of 7 days to 1 year in duration. Alterations in liver weight may suggest treatment-related changes including hepatocellular hypertrophy like enzyme induction or peroxisome proliferation.
Liver weights may be elevated in studies of less than 7 days duration for potent hepatic enzyme-inducing compounds. Elevated heart weight may be the only evidence of myocardial hypertrophy that is often macroscopically and microscopically difficult to recognize (Thiedemann, 1991; Greaves, 2000). Changes in kidney weight may reflect renal toxicity, tubular hypertrophy or chronic progressive nephropathy (Greaves, 2000). Changes in brain weights are rarely associated with neurotoxicity. The utility of brain weight rests in the ability to calculate organ to brain weight ratios. Some consider evaluation of organ to brain weight ratios helpful when terminal body weights are affected by the samples tested or to normalize organ weight data when there is large inter-animal variability. Changes in testes weights may reflect changes in seminiferous tubules or interstitial edema. Changes in epididymal weight may be a sensitive indicator of decreased sperm production or may reflect edema or inflammation (Creasy, 2002). Organ weight changes might represent secondary effects of treatment on the reproductive cycle rather than a direct toxic effect of the sample on test.

The livers of all the mice treated with *Senna spectabilis* and *M. putterlickioides* plant extract had intact hepatocytes, uncongested central veins and sinusoids. However, livers of mice orally treated with an aqueous extract of *O. usambarensis* (1g/kgbw/day) daily for 28 days revealed intact hepatocytes, congested central veins and sinusoids, inflammation around blood vessel and damage at specific points. Histological section of a kidney of mice orally treated with an aqueous extract of *S. spectabilis* and *O. usambarensis* (1g/kgbw/day) daily for 28 days also revealed intense infiltration of inflammatory cells causing necrosis and loss of cellular details.
Alkaloids have been reported to cause liver megalocytosis, proliferation of biliary tract epithelium, liver cirrhosis and nodular hyperplasia (Zeinsteger et al. 2003). This infers that phytochemicals present in the aqueous extracts of *S. spectabilis* and *O. usambarensis* contributed to their antibacterial activity and toxicity to the Albino Swiss mice model.

The level of Lactate dehydrogenase (LDH), Aspartate amino transferase (AST) and Aspartate aminotransferase (AST)/ Alanine aminotransferase (ALT) ratio were high in mice orally treated with an aqueous extract of *O. usambarensis*. LDH is an enzyme found in cells of many body tissues including the heart, liver, kidney, skeletal muscle, brain, red blood cells and lungs. It is responsible for converting muscle lactic acid to pyruvic acid, an essential step in producing cellular energy. High levels of LDH in blood indicate tissue damage. However, isoenzyme tests are better indicators of the specific organ or tissue damage. AST is found in muscle cells and liver cells. ALT is found solely in the liver cells and is useful in detecting liver diseases. The elevated levels of both the enzymes AST and ALT is an indicator of liver damage and is measured together as part of liver function panel to detect liver damage. Liver diseases in which AST is higher than ALT includes alcohol induced liver damage, cirrhosis and liver tumour (Pagana et al., 1998). The plant extract administered to mice at 1g/kg body weight damaged the liver and other body organs. This is in agreement with the increase in size of all the organs except the testis (*O. usambarensis*), increase in size of the kidney, spleen and heart (*S. spectabilis*) and increase in size of kidney, testis and heart (*M. putterlickioides*). This is also supported by the high AST:ALT ratio in all the mice treated with the three plant extracts.
Oral administration of a high dose of *Olinia usambarensis* plant extracts caused microcytic hypochromic anemia. This is indicated by decrease in mean corpuscular volume (MCV) and reduction in packed cell volume (PCV). These abnormal blood conditions could be caused by toxic constituents in the plant extract including among others alkaloids, saponins, flavonoids and tannins present in these extracts. These extract constituents could be reducing the erythron parameters (Barger, 2003).

Saponins hemolyse and cause cell death in many tissues (Al-Sultan *et al.*, 2003). Alkaloids have been shown to cause liver megalocytosis, proliferation of biliary tract epithelium, liver cirrhosis and nodular hyperplasia (Zeinsteger *et al.*, 2003). Terpenoids have been shown to increase membrane permeability to divalent and monovalent ions (Zeinsteger *et al.*, 2003). The trace elements copper and zinc which could also cause toxicity if taken at higher levels were present in levels below the recommended daily allowances. The extract constituents could also cause deficiency of folate through interfering with its absorption or making it biologically unavailable. The condensed tannins reduce feed intake, inhibits digestion and absorption of nutrients such as carbohydrate, essential amino acids and folic acid (Click, 1969).

Hyperchromic anaemia as indicated by high level of MCHC is caused by abnormal concentration of hemoglobin which could be due to over production of smaller than normal reticulocyte as demonstrated by low MCV. This interfere with hemoglobin incorporation in these cells which are larger in size than mature cells resulting in decreased blood flow and poor tissue oxygenation (tissue hypoxia). Tissue hypoxia could be the cause of the increased of organs size. These abnormal tissue sizes could account for increased levels of biochemical parameters such aspartate
aminotransferase and AST:ALT ratio after 28 days treatment with 1g/kg body weight of each of the three plant extracts. Low levels of oxygen causes most tissues such as kidney, liver, heart, brain and testis to initially enlarge and as the swollen cells continue rupturing, the organ size reduces (organ atrophy). As the rate of ATP production decreases below the level required by membrane ion pumps for the maintenance of proper intracellular ionic concentrations, the osmotic balance of the cell is disrupted so that the cell and its membrane enveloped organelles swell.

During tissue hypoxia, cells which rely only on glycolysis for ATP production rapidly deplete the store of phosphocreatine (a source of rapid ATP production) and glycogen. As the rate of ATP production decreases, membrane ion pumps for the maintenance of proper intracellular ionic concentrations are interrupted. This offsets the osmotic balance of the cell such that the cell and its membrane enveloped organelles swell. The overstretched membrane becomes permeable thereby leaking their enclosed contents (Voet et al., 2004). This could explain the increased levels of some of the measured serum biochemical parameters. The mineral content in the plant extract may also cause toxicity to the mice. However, the daily administration of K, Ca, Mn, Fe, Cu, Ti, Ni, and Zn were below the recommended daily allowance. Although there were slightly higher levels of, rubidium, mercury and lead, their toxic effects can only be realize if consumed in larger quantities for a long duration. Thus the quantity of minerals in the medical plant extracts was negligible to cause toxicity.
5.2 CONCLUSIONS


ii) The aqueous medicinal plant extracts of \textit{Senna spectabilis} (Leaves), \textit{Maytenus putterlickioides} (root) and \textit{Olinia usambarensis} (leaves) demonstrated bacteriostatic activity as indicated by their MICs and MBCs. Therefore like chloramphenicol, the extracts do not kill the bacteria but rather inhibit their multiplication.

iii) The phytochemicals present in the three medicinal plant extracts contributed to their activity against diarrhea causing bacteria pathogen as the mineral elements content was in negligible to do so. Thus justifies their continued use in traditional medicine.

iii) Oral administration of 1g/kg body weight of \textit{Olinia usambarensis} (roots) extract to mice on daily basis for one month demonstrated some toxicological effects as evidenced by changes in hematological, biochemical, body weight, organ weights and histological indices. The reduced body weight could be as a result of phytochemicals present in the extract.

5.3 RECOMMENDATIONS

i) This study shows that \textit{Maytenus putterlickioides} (roots) and \textit{Senna spectabilis} (leaves) were active against \textit{Sal. typhi}, \textit{Sh. flexineri} and \textit{Sh. dysenteriae}. I therefore
recommend their use for the treatment of infections caused by these three strains of bacteria at rate of 1g/kg body weight for 7 days.

ii) *S. spectabilis* and *M. putterlickioides* administered orally significantly decreases the number of neutrophils, eosinophils and monocytes in mice thus should not be used for a prolonged period.

iii) *Olinia usambarensis* extracts administered orally causes liver infiltration and venous congestion in mice thus the extract is not safe for use in bacterial infections for a long period.

iv) Oral administration of aqueous extract of *Senna spectabilis* and *O. usambarensis* cause intense infiltration of inflammatory cells causing necrosis and loss of cellular details thus the extracts are toxic to these organs.

v) *Senna spectabilis* and *O. usambarensis* administered orally to mice caused villi disintegration and lack of continuity of the villi coating and thus these extracts are not safe for use due to the toxic effects in the intestines.

vi) The mice were treated with the plant extracts daily for 28 days. However curative treatment of diarrhea causing bacteria infection take 5 to 7 days which means less exposure to these toxic plants. I therefore recommend their continued use.

### 5.4 SUGGESTIONS FOR FURTHER RESEARCH

i) Aqueous extracts are easier to prepare at home but it may be of interest to compare these results with organic solvent extract. Extraction by organic solvents may yield more potent products, as it would improve extraction of active compounds from the plant parts.
ii) There is need to determine the mechanism of antibacterial action of *Senna spectabilis* (leaves), *Maytenus putterlichodes* (roots) and *Olinia usambarensis* (leaves) extracts.

iii) Efforts should be made to isolate and identify bioactive compounds, which are present in the three extracts. This could lead to the identification of new range of compounds for management of bacterial infections.

iv) It is evident that the plants have compounds active against pathogenic organisms. Therefore working relation should be strengthened between traditional healers and scientific institutions to rapidly identify and evaluate these plants.

v) Although some toxicity contraindications were noted, there is need for more toxicity studies to aid in determination of safe regimens if the extracts are to be scientifically encouraged for treatment of bacterial related diarrhea. Also a combined therapy of *Olinia usambarensis* with other plant extracts should be identified to reduce its toxic effects.
REFERENCES


APPENDICES

Appendix 1: Bacteriological Media

Faecal coliforms medium

Medium used for detection and enumeration of faecal coliforms organisms

Formula

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>12.5g</td>
</tr>
<tr>
<td>Bacteriological peptone</td>
<td>10g</td>
</tr>
<tr>
<td>Protease peptone</td>
<td>5g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5g</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>3g</td>
</tr>
<tr>
<td>Bile salts</td>
<td>1.5g</td>
</tr>
<tr>
<td>Aniline Blue</td>
<td>0.1g</td>
</tr>
<tr>
<td>Bacteriological agar</td>
<td>15g</td>
</tr>
</tbody>
</table>

Method

Suspend 52 g of medium in one litre of distilled water. Dissolve until completely dissolved. Add 10ml of rosolic acid at 1% in NaOH 0.2N. Mix well to obtain a homogenous suspension. Heat with frequent agitation till boiling. Cool to 45-50 °C and pour into Petri dish.

ii) Salmonella Shigella Agar

Formula

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Extract</td>
<td>5g</td>
</tr>
<tr>
<td>Peptone</td>
<td>5g</td>
</tr>
<tr>
<td>Lactose</td>
<td>10g</td>
</tr>
<tr>
<td>Bile Salts</td>
<td>8.5g</td>
</tr>
<tr>
<td>Sodium Nitrate</td>
<td>8.5g</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>8.5g</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td>1g</td>
</tr>
<tr>
<td>Bacteriological agar</td>
<td>13.5g</td>
</tr>
<tr>
<td>Neutral Red</td>
<td>0.025g</td>
</tr>
<tr>
<td>Brilliant Red</td>
<td>0.33mg</td>
</tr>
</tbody>
</table>
Method
Suspend 60 mg of medium in one litre of distilled water. Mix well until homogenous suspension is obtained. Heat with frequent agitation and boil for one minute. DO NOT STERILIZE IN AUTOCLAVE. Cool to 45 °C and 50 °C and distribute in Petri plates.
Allow the medium to solidify partially uncovered.
This is a selective media for all isolation of salmonella and Shigella species.

iii) Nutrient gelatin

Formula
Beef extract 3g
Peptone 5g
Gelatin 120g
Dilled water 1000ml

Method
Warm the mixture to about 50 °C to dissolve completely distributes in tubes and sterilize in the autoclave for 15 minutes at 121 °C.

Muller Hinton Medium

Formula
Beef infusion 2g
Casamino acids 17.5g
Starch 1.5g
Agar 17g
Distilled Water 1000ml

Method
Suspend the mixture in cold distilled water and heat to boiling to dissolve the medium completely. Distribute in flask and sterilize for 10 minutes at 116 °C. Cool to 50-55 °C and pour into plates.
Appendix 2: Biochemical tests

ii) Gram stain Formula
Gram’s iodine Weight
Iodine 1g
Potassium iodide 2g
Distilled water 300g

ii) Crystal Violet
Solution A
Crystal violet (90% dye content) 2g
Ethy alcohol (95%) 20ml

Solution B
Ammonium oxalate 0.8g
Distilled water 80ml
Mix the two solutions in the above quantities.
Safranin
Safranin 2.5% solution in 95% alcohol 10ml
Distilled water 100ml

Staining Procedure
1. The heat fixed smear is stained for 1 minute with ammonia oxalate crystal violet.
2. Wash with tap water.
3. Flood with Gram’s iodine solution and allow standing for 1 minute.
4. Wash in tap water and blot dry.
5. Decolorize 30 seconds with gentle agitation in 95% alcohol and blot dry.
6. Counter stain 10 to 30 seconds in safranin.
7. Wash in tap water, blot dry and examine microscopically.
Gram stain is one of the most valuable and generally applied method. It serves to separate bacteria into two groups. Those that retain crystal stain are said to be Gram-positive, while those that are decolorized and stain with counter stain are said to be Gram-negative.
Turbidity Standard

**Formula**

0.045 M Barium chlorides solution
Barium chloride  1.175
Distilled water  100ml

0.36M Sulphuric acid solution
Concentrated sulphuric acid  1 ml
Distilled water  100ml

**Method**

Combine solution 1 and 2
Solution 1  0.5 ml
Solution 2  99.5 ml

Distribute into test tubes closed by rubber stopper. Agitate tube well before use. Store in the dark at room temperature.
Appendix 3: Bacteria identification criteria

**Escherichia coli**: Pink, shiny, mucoid, slightly raised colonies with entire margins, Gram-negative rod, grows on Mackonkey’s, lactose fermenting producing acid, and oxidase negative.

**Salmonella typhi**: Shiny convex colonies with entire margins, Gram-negative rods, Oxidase negative, grows on Mackonkey’s, non-lactose fermenters producing hydrogen sulphide and or G antigens.

**Shigella species**: Gram-negative rods, oxidase negative, grow on Mackonkey’s and non-lactose fermenters.

**Shigella dysenteriae**: Non-motile and anaerogenic

**Shigella sonnei**: Methyl red positive, urease negative, indole negative, ornithine decarboxylase positive.

**Pseudomonas aeruginosa**: mucoid colonies with diffusible green pigmentation, umbonate elevation and characteristic thyl red positive, urease negative, indole negative, ornithine decarboxylase positive. Odour, β-hemolytic, Gram-negative rods, oxidase positive and grow on nutrient agar.

Proteus species: Highly motile on agar plates (with a single bacteria capable of spreading through the surface of agar plate overnight), Gram-negative, oxidase negative, urease splitting, grows on Mackonkey and non-lactose fermenters.

**Proteus vulgaris**: indole positive

**Proteus mirabilis**: indole negative

**Campylobacter jejuni**: small curved or seagull-winged faintly staining Gram-negative rods, oxidase positive, catalase positive, darting motility and curved forms on wet preparation, aerobic and do not grow on nutrient agar.

**Calculation of concentration of Extract on the paper Disc.**

*Calculation of Extract on the paper Disc.*

1000 mg of extract in 4 ml

250 mg of extract in 1 ml

= 250 mg of extract in 1000 µl

*Therefore 5 µl of extract has 5/1000 x 250 = 1.25 mg in the paper disk.*

**Injectable chloramphenicol**

Purchased as chloramphenicol sodium succinate vials (1 gm) a product of Flamingo Pharmaceuticals in Mumbai, India.